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THE EFFECT OF COMBINED A.C. AND D.C. PLATE SUPPLY ON A SHORT WAVE TRIODE OSCILLATOR[1]

BY GEO. S. FIELD[2]

Abstract

By combining A.C. and D.C. on the plate of a triode oscillator, more stable and much stronger oscillations were obtained than with pure D.C. Increases in radio frequency current up to 2.5 times were noted. Experiments with 200-cycle and 60-cycle A.C. showed that the higher frequency gave stronger oscillations.

Introduction

While working with the circuit shown in Fig. 1, using five-watt tubes on a wave-length of about 1.6 metres, an occasion was afforded to modulate the output at a low audio-frequency. For this purpose 100-volt, 200-cycle A.C., was applied to the plate of the tube in series with the normal value of 360 volts D.C.

As a result of this combination, a considerable increase in the antenna current was observed. It was thought that this might be due merely to the increased voltage, which would occur for the peaks, though the average plate voltage would be the D.C. value. If increased voltage were the determining factor, therefore, a direct voltage equal to the peak value of the A.C. plus D.C. should result in even greater radiation than the combination voltage, since the higher voltage is then continuously sustained instead of being reached only momentarily 200 times a second. Accordingly a direct voltage was

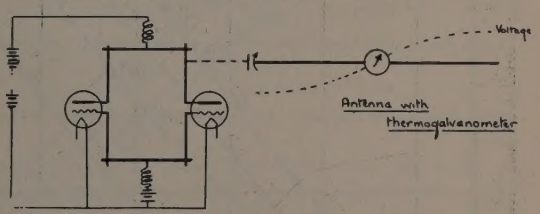

FIG. 1. *Electrostatic coupling circuit.*

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applied to the plate equal to the peak voltage previously used. The resulting antenna current was not as great as before, instead of being greater.

It has been known amongst radio engineers for some time that modulating the plate voltage will cause stronger oscillations to be produced. For communication purposes, however, modulated C.W. is objectionable, on account of the resulting side bands (1) and the interference which is therefore likely to occur with other signals.

For very short wave-lengths (below five metres), however, in most cases this objection is not an important one, and since the production of these short waves presents considerable difficulties, it seems well worth while to take advantage of a method which does give such a big increase in the strength of the oscillations.

Although, as is mentioned above, the effect described here is not a new one, so far as the writer is aware no quantitative results have been published in this connection. It is hoped then that it will be of some interest to know more definitely the increase in output to be expected when A.C. and D.C. are combined for the plate supply.

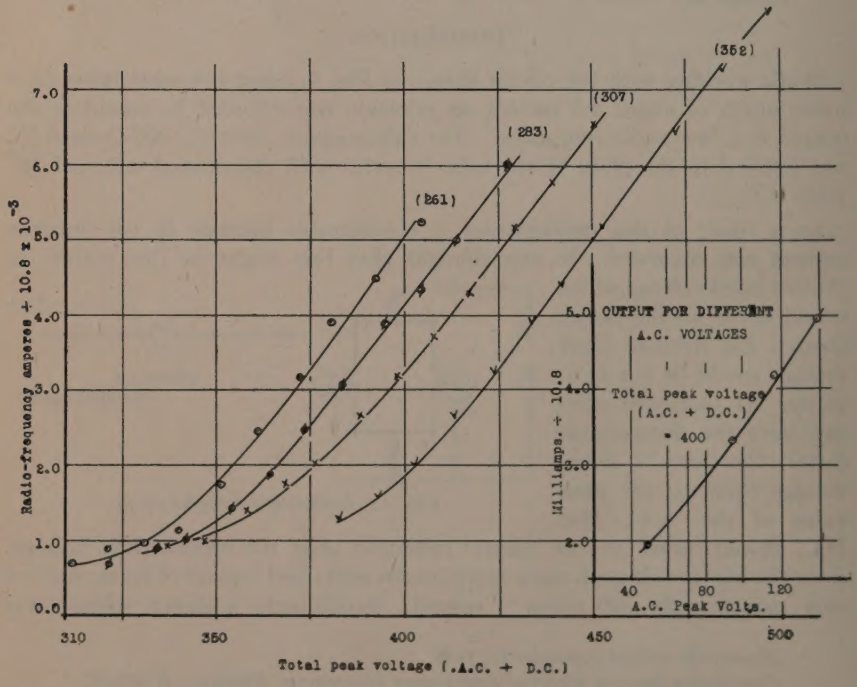

FIG. 2. *Curves showing effect of combining A.C. and D.C. for the plate supply of a triode oscillator.*

Experimental

Following the observations noted above, a number of readings were taken of antenna current corresponding to different combinations of A.C. and D.C. plate supply. The results obtained are represented graphically in Fig. 2. These curves represent 261-volt constant D.C., plus different peak values of A.C., 283-volt D.C., plus different values of A.C., and so on.

An additional curve has been plotted showing the effect of different proportions of alternating voltage on the antenna current, the total peak voltage being kept constant at 400.

These graphs indicate that a combination of A.C. and D.C. plate supply produces considerably stronger oscillations than does pure D.C. It also appears that the greater the proportion of A.C., the stronger the oscillations. Whether or not a maximum might occur in the curve of radiation against different proportions of A.C. is not yet known, as with the voltages available at the time it was not possible to produce the curve any further than is shown. It should be pointed out, too, that the D.C. voltage was always in excess of the A.C. voltage, so that the total plate voltage was always positive. If the proportion of A.C. were increased so that the voltage minima were negative, a dropping off in radiation might be expected; but further experimentation will be necessary to decide this point.

The effect of changing the frequency of the A.C. applied to the plate of the triode oscillator was also studied. Two different frequencies were combined with the direct current, *i e.*, 200 and 60 cycles. The results, plotted in Fig. 3,

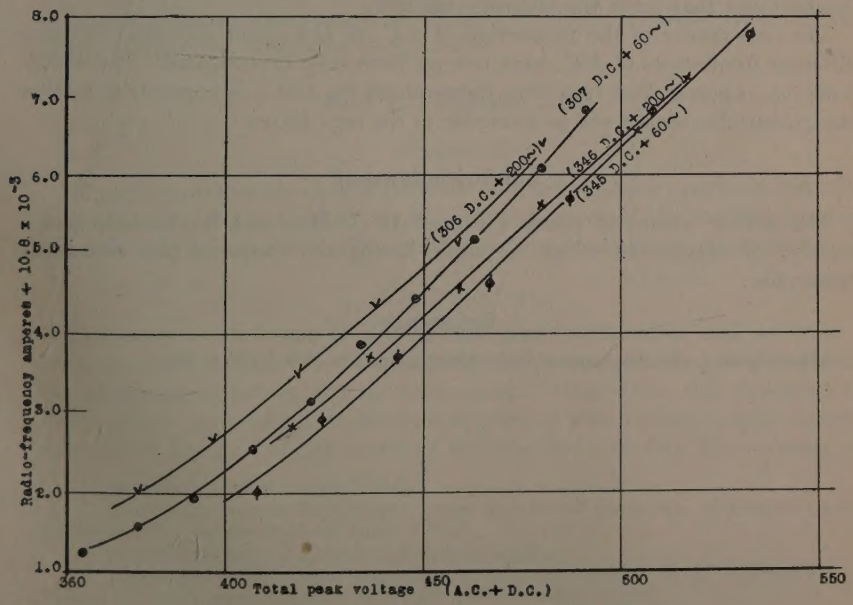

FIG. 3. *Effect of changing frequency of A.C. applied to plate of triode oscillator.*

indicate that frequency may be of importance in obtaining strong oscillations, but a greater range of frequencies should be tried to investigate this possibility.

To bring out the effect of combination plate supply in a slightly different way, the dimensions of the oscillator were reduced till with pure D.C. on the plate the circuit would not oscillate at all. Upon using a combination, however, strong oscillations again occurred.

It was observed, too, that even when oscillations could be produced with D.C. alone, they were not as stable as with D.C. plus A.C. That is, any disturbing influence in the proximity of the oscillator, such as a wave-meter or the human hand, would cause the plate milliammeter to go up as much as 10 milliamperes, and sometimes cause oscillations to cease entirely. With D.C. plus A.C., however, such influences affected the meter by only a milliampere or so, and the oscillations were not easily damped out.

Conclusions

It has been shown that a combination of A.C. and D.C. on the plate of a triode oscillator is much more effective in producing very short waves than is pure D.C. This effect is quite marked, as may be seen from Fig. 2. For instance, the increase in radio-frequency current produced by increasing the A.C. peak voltage from about 50 to about 140 (total peak voltage constant at 400) was in the ratio of 5/2 or an increase in output energy in the ratio of 25/4.

In addition to the increase in the strength of the oscillations, a considerable increase in stability was noted, and it is possible to go to lower limits with this combination than with the ordinary method.

The importance of the proportion of A.C. to D.C., and the effect of using different frequencies of A.C. have not yet been fully investigated. The writer, however, is going more fully into these questions, and it is hoped that further experimental evidence will be available in the near future.

Acknowledgment

The author wishes to thank Professor H. J. MacLeod for his help and a number of suggestions which he made during the course of this and other researches.

Reference

1. MORECROFT, J. H. Elements of Radio Communication, New York, p. 185.

THE OXIDATION OF SOME DIBASIC ACIDS[1]

By W. H. Hatcher[2] and W. H. Mueller[3]

Abstract

This paper gives the results obtained when hydrogen peroxide is employed to oxidize malonic, tartronic, succinic, malic, tartaric, maleic and fumaric acids. The rate of reaction for each has been determined and compared with previous findings for other compounds. The mode of oxidation suggests in each case a complex through which decomposition occurs; the rates of reaction indicate the comparability of saturated acids having the same number of carbon atoms, the constancy of mono-hydroxylization in its velocity influence, and the diverse effects of hydrogen ion concentration. The effects of geometrical isomerism and the ethylenic linkage are well-marked. The formation of peracids is to be regarded in the nature of a side-reaction in these oxidations.

Introduction

Previous experimentation with the simpler aldehydes and acids has demonstrated the role which hydrogen peroxide plays in their oxidation. The results obtained in the present research show many similarities to former observations and deal with the relative rate and mode of oxidation of dibasic acids and the influence of concentration and hydrogen ion on such rates. As formerly, attempts have been made to produce whatever similarities exist between hydrogen peroxide and potassium permanganate as oxidizing agents.

For convenience this paper is divided into parts according to the acid studied, such parts containing necessary references and experimental details; the conclusions are kept till the end so that the results obtained here can be correlated with such previous observations as will tend to throw most light on the general mode of oxidation of organic compounds.

Part I. Malonic Acid

Historical

Though no previous hydrogen peroxide data are available, yet with potassium permanganate, formic acid has been identified as one of the reaction products (6), and it has been postulated that two molecules of the latter are obtained (14).

Experimental

The material used was of the highest grade obtainable, and no trace of impurity could be detected. Preliminary tests with hydrogen peroxide showed the formation of formic but no acetic acid. These tests also indicated the most suitable concentrations and temperature at which a convenient measurement could be made of the course of the reaction. In this, as in subsequent

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Contribution from the Department of Chemistry, McGill University. Presented as partial fulfilment of the requirements for the degree of Ph.D.

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cases, the rate of oxidation was determined by measuring the carbon dioxide evolved from a mixture of the reagents, in the apparatus previously employed; this apparatus was identical with that described elsewhere (11, 12) except that a spiral condenser surrounded by an ice-salt mixture was used to trap any oxidation products other than carbon dioxide. Every precaution was taken to ensure such accuracy as was possible. In all these reactions 100° C. was the temperature employed, except where otherwise stated.

Table I contains the results of an experiment designated "standard" for comparison with other compounds similarly treated.

TABLE I

OXIDATION OF MALONIC ACID

Reagent	Concentrations	
	gm.	Per cent
Malonic acid	1.6278	3.26
Hydrogen peroxide	2.5000	5.00
Water		91.74
Time in min.	**Per cent malonic acid oxidized**	**$K \times 10^4$**
44	2.0	2.0
89	7.3	3.7
152	15.5	4.8
212	21.6	5.0
272	27.0	5.0
332	31.0	4.9
389	34.5	4.7

NOTE:— *Molecular ratio 1:4.7; Temperature = 100° C. Percentage hydrogen peroxide used up 83.1.*

At the conclusion of the experiment the mixture remaining in the reaction flask was analysed for hydrogen peroxide. Another series of observations was made at identical time intervals on a similar mixture to find the rate at which the hydrogen peroxide was disappearing; the method was to pipette an aliquot and add it to acidulated potassium iodide solution, subsequently titrating the iodine liberated. Thus with the loss of both organic substance and hydrogen peroxide, these values were applied in the equations for mono- and dimolecular reactions. The values given in the third column of Table I are obtained using the monomolecular equation and show fairly good agreement after the reaction has once begun.

Influence of Concentration

Malonic acid	7.68%
Peroxide	10.00%
Molecular ratio	1.9 : 9.4

In 365 min. 37.9% of the acid had been oxidized to carbon dioxide.

Influence of Mineral Acid

Malonic acid	7.68%
Peroxide	5.00%
Hydrochloric acid	0.037%
Molecular ratio	1.9 : 4.7

The constants calculated as before ranged from 3.8 to 3.5×10^{-4} in 414 min., 90.1% of the peroxide having disappeared.

Reaction with Di-sodium Malonate

With sodium malonate and hydrogen peroxide present in concentrations similar to those in Table I, only 1.1% oxidation to carbonic acid resulted in two hours, at the end of which time all the peroxide had disappeared.

Part II. Tartronic Acid

Historical

Fenton and Jones (8) obtained mesoxalic acid by the action on tartronic acid of hydrogen peroxide in the presence of ferrous iron.

Experimental

The material used here was prepared from malonic acid, according to the method of Wagner (18), with this exception that hydrolysis of the monobrom-malonic acid was brought about by baryta water; the resulting barium tartronate was treated quantitatively with dilute sulphuric acid to obtain solutions of the free tartronic acid.

Oxidation with Permanganate

As the reaction of permanganate on tartronic acid had not previously been studied, mixtures were made up according to a scheme previously followed (14) and the loss of permanganate estimated at 25° C. at various intervals.

Fig. 1 gives the results obtained from mixtures made up to have the following concentrations; these are given according to the curves found in the figure. The total volume of each mixture was 250 cc.

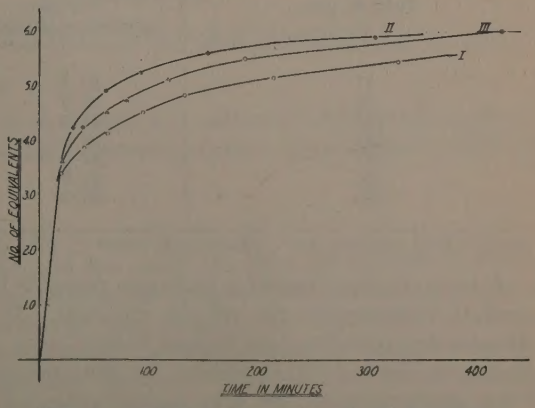

FIG. 1. *Oxidation of tartronic acid with potassium permanganate.*

TABLE II

VARIOUS CONCENTRATIONS OF TARTRONIC ACID USED

Reagent	Curve I	Curve II	Curve III
Tartronic acid	0.1553 gm.	0.1869 gm.	0.1647 gm.
Permanganate	0.0626 *N.*	0.1492 *N.*	0.1224 *N.*
Sulphuric acid	0.0522 *M.*	0.0522 *M.*	0.1305 *M.*

A consideration of these curves, whose ordinates represent the number of equivalents of permanganate used up during the oxidation of one gram-molecule of tartronic acid, will show that one gram-molecule of formic acid is at first split off: this is slowly oxidized to its end products. Also increased acidity encourages the oxidation of tartronic acid, a point of importance in permanganate oxidations.

Oxidation with Peroxide

Preliminary experiments showed formic acid as the only identifiable product of such reaction. As in the case of malonic acid, a reaction mixture was made up and the evolution of carbon dioxide followed with time. The details of this experiment follow.

TABLE III

OXIDATION OF TARTRONIC ACID

Reagent	Concentrations	
	gm.	Per cent
Tartronic acid	1.8763	3.75
Hydrogen peroxide	2.5000	5.00
Water		91.25
Time in min.	Per cent tartronic acid oxidized	$K \times 10^4$
51	10.2	9.1
86	16.9	9.3
143	24.9	8.7
203	29.6	7.5
263	34.2	6.9
324	37.0	6.2
386	39.3	5.6

NOTE:– *Molecular ratio 1:4.7; Temperature = 100° C. Percentage hydrogen peroxide used up 84.2*

A separate experiment for hydrogen peroxide loss with time was made, and applied to determine the velocity constant. In neither the mono- nor the dimolecular equation (the former values being given in Table III) did the constants show good agreement, as the same falling-off was noted. Using other concentrations, the time to half-value was found; here the agreement pointed to the reaction being mainly monomolecular.

Influence of Concentration

Tartronic acid	5.75%
Peroxide	3.79%
Molecular ratio	1 : 2.35

In 297 min. 15.3% of the acid had been oxidized with a peroxide loss of 71.3%; the constants varied from 3.5 to 2.4×10^{-4}.

Influence of Mineral Acid

Tartronic acid	3.75%
Peroxide	5.00%
Hydrochloric acid	0.037%
Molecular ratio	1 : 4.7

The constants ranged from 7.3 to 5.9×10^{-4} in 220 min.

Reaction with Di-potassium tartronate

With concentrations similar to those given in Table III, all the hydrogen peroxide had disappeared in two hours, and only 2.7% of the tartronate had been oxidized to carbonic acid.

Part III. Succinic Acid

Historical

Oxalic, tartaric and malic acids have been identified (1, 2, 17, 21) as oxidation products of succinic acid, though most of the experimentation has been of a biological nature. No data on either acid permanganate or hydrogen peroxide in this case are available.

Experimental

The succinic acid used was found to be free of all impurities.

Oxidation with Permanganate

Under conditions similar to those used with tartronic acid, succinic acid was found to be unattacked by potassium permanganate in the presence of sulphuric acid.

Oxidation with Hydrogen Peroxide

In preliminary tests formic acid was easily identified, as was also a trace of acetaldehyde. The following table gives details of the standard experiment where the progress of the reaction was measured by the weight of carbon dioxide evolved. The figures given in the third column of Table IV are obtained by using the monomolecular equation. The loss of hydrogen peroxide alone was followed in a separate experiment at the same time intervals. It will be observed that the constants after the first interval are well sustained.

TABLE IV

OXIDATION OF SUCCINIC ACID

Reagent	Concentrations	
	gm.	Per cent
Succinic acid	1.8468	3.69
Hydrogen peroxide	2.5000	5.00
Water		91.31
Time in min.	**Per cent succinic acid oxidized**	**$K \times 10^4$**
61	2.5	1.8
137	7.7	2.6
209	12.4	2.7
266	15.0	2.7
328	17.6	2.6
385	19.8	2.5

NOTE:– *Molecular ratio 1:4.7; Temperature = 100° C. Percentage hydrogen peroxide used up 63.9.*

Influence of Concentration

Succinic acid 3.69%
Hydrogen peroxide 10.00%
Molecular ratio 1 : 9.4

During six hours the constants calculated as before ranged from 2.9 to 3.0×10^{-4}. This increase due to concentration is much smaller than usually encountered with hydrogen peroxide reactions; at the end of 380 min. 61.0% of the peroxide had been used up.

Influence of Mineral Acid

The concentrations were the same as in the standard experiment except for the hydrochloric acid, present to the extent of 0.037%. For a period of 6.5 hr. the constants ranged from 2.2 to 2.1, *i.e.*, centinormal acid caused a slight decrease in the rate of oxidation of the succinic acid, the peroxide suffering 64.8% decomposition.

Influence of the Sodium Ion

Using the di-sodium salt instead of the free acid, and with concentrations similar to those used in the standard experiment, rapid oxidation ensued for the first few minutes; this soon decreased so that after 155 min. only 3.4% of the succinate was oxidized. At this time no hydrogen peroxide remained.

Part IV. Malic Acid

Historical

Using hydrogen peroxide and ferrous sulphate, Fenton (7) obtained oxalacetic and pyruvic acids from malic acid, while without a catalyst Zinno reported *d*-tartaric acid (20). Walton and Graham (19), using Fenton's method, showed that the introduction of an hydroxyl group into the succinic acid molecule increases the rate of oxidation.

With potassium permanganate in acid solution two molecules of formic acid are produced (14).

Experimental

Preliminary tests on a very pure sample of malic acid showed that acetaldehyde is obtained with peroxide; attempts to determine the amount, however, failed. Also, traces of acetic acid were found. Formic acid was easily identified among the reaction products.

In Table V appear the concentrations and results of the standard experiment.

TABLE V

Oxidation of malic acid

Reagent	Concentrations	
	gm.	Per cent
Malic acid	2.0962	4.19
Hydrogen peroxide	2.5000	5.00
Water		90.81
Time in min.	**Per cent malic acid oxidized**	**$K \times 10^4$**
30	3.4	5.0
60	12.4	9.6
115	21.1	9.3
175	28.8	8.4
235	34.2	8.2
295	37.6	7.0

Note:– *Molecular ratio 1:4.7; Temperature = 100° C. Percentage hydrogen peroxide used up 85.1.*

As in previous cases the loss of hydrogen peroxide was followed quantitatively; the constants above are calculated from the monomolecular equation, where the greatest agreement was found.

Influence of Concentration

Malic acid	4.19%
Hydrogen peroxide	10.00%
Molecular ratio	1 : 9.4

For time intervals similar to those in Table V the constants were 6.1, 10.9, 11.1, 10.6, 9.9, 9.1×10^{-4}.

Influence of Mineral Acid

With concentration as in Table V except for the presence of hydrochloric acid to the extent of 0.037%, the rate was almost exactly 50% of that obtained in the standard experiment.

Influence of Potassium Ion

Using the di-potassium salt with concentrations similar to those of the standard, it was found that only 3.9% of the succinate had been oxidized in 205 min.

Part V. Tartaric Acid

Historical

Fenton (7) used tartaric acid with hydrogen peroxide and ferrous sulphate for the preparation of dihydroxy maleic acid. It has been established that acid permanganate gives rise to two molecules of formic acid (14), while both formic acid and glyoxal have been found as products of the oxidation with oxygen (3).

Experimental

Preliminary experiments, using dilute hydrogen peroxide and a very pure sample of *d*-tartaric acid, gave definite evidence of formic acid but of no oxalic.

The following table shows the results of the standard experiment, where the reaction is followed by weighing the carbon dioxide evolved.

TABLE VI

Oxidation of tartaric acid

Reagent	Concentrations	
	gm.	Per cent
Tartaric acid	2.2348	4.47
Hydrogen peroxide	2.5000	5.00
Water		90.53
Time in min.	**Per cent tartaric acid oxidized**	**$K \times 10^4$**
68	5.5	3.6
104	9.6	4.2
138	13.6	4.6
197	19.8	4.9
266	26.4	5.0
329	31.9	5.1
372	35.2	5.1

Note:– *Molecular ratio 0.95:4.7; Temperature = 100°C. Percentage hydrogen peroxide used up 60.8.*

In a separate experiment the loss of hydrogen peroxide was followed at the same intervals. The constants calculated from these figures were found to agree best from the monomolecular equation and these are given in Table VI.

Influence of Concentration

Tartaric acid	4.47%
Hydrogen peroxide	10.00%
Molecular ratio	0.95 : 9.4

In 378 min. 46.6% of the tartaric acid was oxidized to 49.6% loss of hydrogen peroxide. The constants calculated as before gave values rising from 4.9 to 7.2.

Influence of Mineral Acid

With concentrations as in Table VI except for the addition of hydrochloric acid to the extent of 0.037%, 24.7% of the tartaric acid had been oxidized in 375 min. with a loss of 50.2% of hydrogen peroxide. The constants rose from 1.1 to 3.3.

No attempt was made to study the effect of alkali metal ion on the reaction because of the extreme difficulty in preparing a pure neutral tartrate suitable for use.

Part VI. Maleic Acid

Historical

Meso (15) and racemic (16) tartaric acids have been frequently found as oxidation products of maleic acid. In addition, glyoxylic (9) and formic (14) acids have been found; the only data on the reaction with hydrogen peroxide are available from contemporaneous researches, where it was definitely shown that no addition had taken place at ordinary temperatures across the ethylene linkage (13).

Experimental

Preliminary tests with very pure maleic acid produced formic acid, but it was found impossible to identify glyoxylic or oxalic acid; similarly tartaric acid was unidentifiable.

The following table contains details of the standard experiment where the carbon dioxide evolved was weighed at intervals.

The loss of peroxide was followed in a separate experiment at the same intervals as shown in Table VII and constants calculated as before; those given in the table are from the monomolecular equation and show best agreement.

Influence of Concentration

Maleic acid	3.63%
Hydrogen peroxide	3.19%
Molecular ratio	1 : 3

In 396 min. 34.4% of the maleic acid and 72.2% of the peroxide had been used up. The constants calculated as before for intervals from 107 to 396 min. ranged from 3.3 to 4.8.

TABLE VII

OXIDATION OF MALEIC ACID

Reagent	Concentrations	
	gm.	Per cent
Maleic acid	1.8152	3.63
Hydrogen peroxide	2.5000	5.00
Water		91.37
Time in min.	**Per cent maleic acid oxidized**	**$K \times 10^4$**
40	4.8	5.4
82	15.3	8.9
133	26.0	9.8
200	36.4	9.8
263	45.3	10.0
323	52.4	10.0
385	57.0	9.5

NOTE:– *Molecular ratio 1:4.7; Temperature = 100° C. Percentage hydrogen peroxide used up 80.5.*

Influence of Mineral Acid

Maleic acid	3.63%
Hydrogen peroxide	3.73%
Hydrochloric acid	0.036%
Molecular ratio	1 : 3.5

In 43 min. 14.7%, and in 85 min. 27.9% of the maleic acid had been oxidized. The constants calculated as before were 16.1 and 16.7. Maleic acid is thus one of the few substances whose oxidation is increased by the addition of mineral acid.

Influence of the Sodium Ion

Using di-sodium maleate and hydrogen peroxide of concentrations similar to those of Table VII, 3.2% of the maleate and all the peroxide had disappeared in 120 min.

Part VII. Fumaric Acid

HISTORICAL

Racemic and formic acids have been identified in the permanganate oxidation of fumaric acid; in acid permanganate solution there is very little difference between fumaric and maleic acids, each producing one molecule of formic acid (14).

Experimental

In the preliminary tests only formic acid was identified, no oxalic or glyoxylic being found.

In the standard experiment, as detailed in Table VIII, all the fumaric acid did not go into solution for nearly seven minutes. Though a smaller quantity would have been soluble yet it was desired to have the same molecular concentration as was employed in the other cases. Consequently the reaction was not quite comparable for the first interval; but in nearly all these cases where the reaction is followed by measurement of carbon dioxide evolution, a longer or shorter period of induction is always observable.

TABLE VIII

Oxidation of fumaric acid

Reagent	Concentrations	
	gm.	Per cent
Fumaric acid	1.8152	3.63
Hydrogen peroxide	2.5000	5.00
Water		91.37
Time in min.	**Per cent fumaric acid oxidized**	**$K \times 10^4$**
90	4.6	2.2
147	10.8	4.1
203	18.3	4.3
266	28.2	5.4
316	34.5	5.8
376	38.9	5.7

Note:– *Molecular ratio 1:4.7; Temperature = 100° C. Percentage hydrogen peroxide used up 62.6.*

As previously the loss of peroxide with time was followed, and these values used to calculate the constants; those in the table above are from the monomolecular equation. The gradual increase in the values has been frequently encountered and considered previously. The chief point of interest lies in the fact that they are just half of those obtained with maleic acid.

Influence of Concentration

Fumaric acid	3.63%
Hydrogen peroxide	2.50%
Molecular ratio	1 : 2.35

After 395 min. 21.5% of the acid and 47.9% of the peroxide had disappeared. The constants ranged between 2.2 and 2.7 for the last 300 min.

Influence of Mineral Acids

With concentration as in the preceding paragraph, except for the presence of hydrochloric acid to the extent of 0.036%, the results obtained were almost

identical with those without additional acid. Thus in 348 min., 18.1% of fumaric acid and 45.0% of peroxide had disappeared; also the constants ranged from 2.2 to 2.5 over a similar range.

Influence of Sodium Ion

With concentrations similar to those used to determine the influence of concentration, di-sodium fumarate was oxidized to the extent of 5.8% in 91 min., four-fifths of this occurring in the first half-hour. Subsequent analysis showed the total loss of the peroxide. Differing from previous cases mentioned here, the reaction at first was twice as rapid with the sodium salt of fumaric as with that of any other acid. Undoubtedly if the hydrogen peroxide had not disappeared so rapidly due to catalytic effects, sodium fumarate would be much more quickly oxidized than the free acid itself.

Part VIII. Peracids

Historical

The changes which result from mixing organic acids with aqueous hydrogen peroxide have been studied heretofore in several cases and have in many instances shown quantitatively the production of peracids at such temperatures as would not involve disruptive oxidation of the organic molecule (11, 12). It has been amply demonstrated previously that the following equilibrium results:

$$RCOOH + H_2O_2 \rightleftarrows RCOOOH + H_2O.$$

The method of following this production of peracid to the establishment of equilibrium consists in making a suitable aqueous mixture at 0° C., and from time to time testing aliquots by addition to a very dilute aqueous solution of neutral potassium iodide; the presence of peracid (as distinct from organic peroxide or hydrogen peroxide) is indicated by an immediate liberation of iodine: the latter is then rapidly titrated with $N/70$ sodium thiosulphate.

This method was employed with the acids studied in the former sections, but difficulties due to insolubility and liability to react oxidatively eliminated tartronic, succinic and fumaric acids.

The following table provides selected data showing the concentrations of acid and hydrogen peroxide and resulting peracid together with the time required for the establishment of equilibrium.

TABLE IX

Formation of peracids

Acid	Conc. of acid in %	Conc. of peroxide in %	Conc. of peracid in %	Time to equilibrium
Malonic	22.4	7.4	0.4	240 hr.
Malic	32.2	8.3	0.6	24 hr.
Tartaric	32.0	7.3	0.2	24 hr.
Maleic	21.5	8.9	2.4	170 hr.

Note:– *The above values are correct to 10% except in the case of maleic acid, where the accuracy is nearer to 4% of the actual.*

Taking these data in conjunction with those previously obtained, it is found that a monobasic acid forms by this procedure more than twice as much peracid as a dibasic one, maleic acid providing an exception. Only a trace of succinic peracid was observable; if the solubility of succinic acid had sufficed for comparison, its peracid value would have been greater than that of tartaric but less than that of malic. Previous data show that an α-hydroxy acid always gives less peracid than the hydrogen acid; examples of this are acetic and glycollic, and propionic and lactic acids.

Succinic peracid was prepared by Clover and Houghton (4) from succinyl peroxide and water. They heated this peracid and found it to give β-hydroxy propionic and later acrylic acid.

From all the observations of oxidation and peracid formation, it is concluded that the latter is not a step in the oxidative decomposition of an organic acid, but merely a side issue; there is no quantitative relationship amongst the rate of oxidation, percentage of peracid formed and dissociation constant of any one acid. It is curious, however, that there is a rough agreement, not susceptible as yet to mathematical expression, between the oxidizability of an organic acid and the time taken for establishment of equilibrium in the equation given above.

Discussion of Results

The foregoing experimental data, which have been briefly presented, indicate the formation of a complex between the organic acid and hydrogen peroxide; contemporaneous results in course of publication indicate from conductivity measurements that this formation is a rapid one, not however to be confused with peracid formation which is relatively a slow process. Orientation of this complex results in the formation, in the cases under consideration, of much less stable bodies whose oxidation proceeds too rapidly to admit of identification of anything but carbonic or formic acids. The occasional occurrence of traces of acetaldehyde has been observed in many inexplicable instances by Denigès (5) the obvious conclusion being that such formation is the result of a minor side reaction which plays no role of importance in the general scheme.

Certain conclusions from this and previous work are easily noted; *e.g.*, omitting that part of the reaction between acid and peroxide represented by the period of induction and remembering that hydrogen peroxide is continually being lost to the reaction catalytically, it is seen that an α-hydroxy acid is just twice as rapidly oxidized as the hydrogen acid; that the rates of oxidation of each of the following sets are comparable: malonic and propionic; succinic and *n*-butyric; tartronic, malic and maleic.

These observations lead to the conclusion, when taken with the results of preliminary tests, that the oxidation of malonic acid proceeds through tartronic to glyoxylic and formic acids, the second named going rapidly to formic acid and ultimately carbonic acid. Mesoxalic, which does not give rise to formic acid, is therefore eliminated.

The oxidation of tartaric acid is just twice as rapid as that of succinic,—a characteristic of α-hydroxy acids which split off formic acid,—the latter and the residue being oxidized at their own rates which are relatively high. It

does not seem probable that (as with Fenton's reagent) tartaric gives rise to dihydroxy maleic acid, since the latter adds on hydrogen peroxide giving dihydroxy tartaric and later oxalic acid quantitatively (13). Tartaric thus behaves as an α-hydroxy acid.

Malic acid on the other hand is about three times as rapidly oxidized as succinic acid; this places it in the category of the β-hydroxy acids. Such would involve the formation of oxalacetic acid; however, the latter would by all previous analogies give rise to malonic acid, whose rate of oxidation is too slow to be included in the disintegration of malic acid. The following scheme would be satisfactory; unfortunately, however, too little is known of the intermediates represented to state definitely that there is no other scheme:

$$COOH.CHOH.CH_2.COOH \leftrightarrows COOH.CO.CH_2.COOH$$

$$\leftrightarrows COOH.COH = CH.COOH \leftrightarrows COOH.CO.CHOH.COOH.$$

The last compound, by analogy with dihydroxy maleic acid would give rise to tartronic acid whose rapidity of oxidation and formation of formic acid would suit the rate of oxidation of malic acid.

Succinic acid might be considered to give rise first to malic acid; but the latter does not give rise to tartaric acid. In the light of the scanty literature, it may do as suggested, or double hydroxylization to tartaric acid might be involved in one step; the latter is more favored by the authors.

Maleic and fumaric acids provide very considerable contrast, the former being twice as rapidly oxidized as the latter. Contemporaneous studies incline against the belief that maleic acid adds on hydrogen peroxide. Fumaric acid alone shows no velocity change in the presence of mineral acid whereas maleic acid has its rate more than doubled under identical circumstances. This fact aligns maleic with oxalic and *n*-butyric acids, the latter undergoing "ketone hydrolysis" in its later stages of oxidation. Maleic shows about the same rate of oxidation as malic, and the rising constant of fumaric suggests the addition of hydrogen peroxide to give rise to tartaric acid. If the latter occurs, it would be in line with known reactions. In the light of much data it is suggested that fumaric acid behaves in this manner, and that maleic acid is mono-hydroxylated, the resulting compound proceeding as given under malic acid.

It is peculiar that though tartaric acid represents the highest condition of oxidation of the compounds studied yet its rate of oxidation lies between succinic and malic. The effect of substituting the potassium or sodium salts for the acids shows that not only is most of the hydrogen peroxide lost catalytically, but the salts themselves in the more or less ionized state are not good subjects for hydrogen peroxide oxidation. Other data (10) support this strongly, indicating that whatever oxidation takes place is due to nascent or molecular oxygen and not to the peroxide molecule itself. On the other hand, the presence of hydrogen ions, brought about by the addition of centinormal hydrochloric acid, causes in only one known case no change whatever in the rate of oxidation, *i.e.*, fumaric acid. Where decrease in rate results to the extent of about 30% we have as examples malonic, succinic and tartronic acids; where the decrease is 50% we find malic and tartaric acids; and where there is

a definite increase maleic acid represents it to the extent of nearly 100%. Former investigations add others to the above three classes: to the first, formic, pyruvic, mesoxalic, and propionic acids; and to the third oxalic, glyoxylic, butyric and isobutyric acids. As previously indicated some acids have their rates of oxidation definitely retarded and others increased by potassium permanganate due to variation in the hydrogen ion concentration. Hydrogen peroxide strongly resembles permanganate in this particular, though there are a few cases where analogous reactions do not occur. Oxidizability is therefore not due to any one of the following factors but to a combination of two or all three of them: susceptibility to oxidation of the organic compound, reactivity of hydrogen peroxide to the kind of structure presented to it, and ionic condition of the medium. It is of interest that maleic and fumaric acids, so different in their actions towards hydrogen peroxide, behave with acid potassium permanganate in an almost identical manner (14).

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STUDIES ON REACTIONS RELATING TO CARBOHYDRATES AND POLYSACCHARIDES

XXXII. THE CONSTITUTION OF SEDOSAN (ANHYDRO-SEDOHEPTOSE)[1]

By Harold Hibbert[2] and C. G. Anderson[3]

Abstract

Methylation of sedosan (anhydro-sedoheptose) gives a tetramethyl sedosan, which on oxidation by nitric acid, yields an optically inactive trimethoxy glutaric acid. By esterifying the latter, and treating the ester with anhydrous methylamine, an optically inactive methylamide, presumably trimethoxy riboglutaro-dimethylamide is obtained. No trace of the methylamide of inactive dimethoxy succinic acid could be found. It is thus concluded that sedose possesses a 2:6 oxygen ring and that sedosan is an anhydro-sedopyranose.

This is confirmed by the isolation of monotrityl sedosan, which shows that sedosan has only one primary alcohol group. Sedosan is therefore 2:7-anhydro-sedopyranose.

The epimerisation of trimethyl δ-arabonolactone into trimethyl δ-ribonolactone apparently takes place only with great difficulty.

The structure of sedosan is found to be in harmony with its lack of tendency towards polymerization, and this is in accordance with the views of Hibbert on the relation of structure to polymerization.

Introduction

In preceding communications (7, 8, 9) on the nature of the changes involved in the synthesis of polysaccharides, the important role played by the hydroxyl groups in the γ:δ-positions of a γ:δ-dihydroxy carbonyl derivative was pointed out. Evidence was brought forward to show that such products possess a pronounced general tendency readily to undergo loss of a molecule of water, especially under the influence of a trace of acid. The molecule of water thus removed is formed as a result of the interaction of the oxygen of the carbonyl group and the hydrogen atoms attached to the γ- and δ-hydroxyl groups respectively, and the resulting product is characterized by a remarkable tendency to undergo an immediate transformation into a complex polymerized derivative. This phenomenon is due presumably in such cases to the inability, owing to spatial relations, for ring closure to occur with formation of the monomolecular anhydro-sugar in the form of a bicyclic acetal.

The carbohydrate, sedoheptose, offers considerable interest from this point of view inasmuch as it readily forms, under the influence of dilute acids, an equilibrium mixture of the stable, free sugar and a crystalline anhydride.

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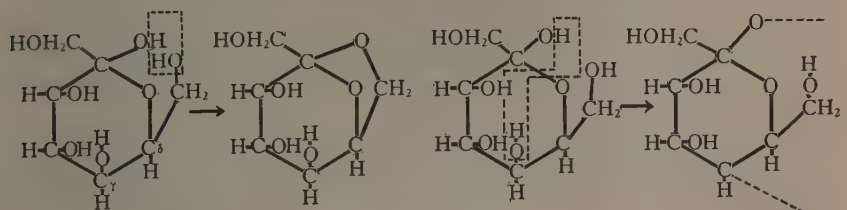
This anhydride which was isolated by La Forge and Hudson (12) from its benzylidene derivative (the latter prepared by the aid of 70% sulphuric acid), is stable towards acids, alkalis and water and shows no tendency to undergo polymerization even under the influence of relatively concentrated sulphuric acid. This lack of tendency towards polymerization would seem to indicate that "sedoheptose anhydride" formation probably does not take place through the medium of γ:δ-hydroxyl groups, since in these cases the formation of a higher polymer would be expected.

Due to the brilliant work of Haworth and coworkers it has now been definitely established that the "normal" form of pentose and hexose derivatives possesses the "pyranose" ring structure. It thus seemed highly probable that sedoheptose in solution exists largely in this form and that anhydride formation probably takes place through the removal of the elements of water from the aldehydrol group and the H atom of the ϵ-hydroxyl group, thus giving rise to a relatively strainless 2:7-anhydro-pyranose derivative. This would account for the lack of tendency to polymerize which previous work has shown would be expected, were the condensation to occur through the γ:δ-hydroxyl groups.



No tendency to polymerize *Complex polymer*

The investigation described below has confirmed the truth of this theoretical viewpoint in that it establishes the structure of sedoheptose anhydride as a 2:7-anhydro-pyranose derivative.

Constitution of Sedosan (Anhydro-sedoheptose)

Sedoheptose, or, as it may be called more conveniently, sedose, was isolated by La Forge and Hudson (12) by extracting the leaves and stalks of *Sedum spectabile* with water. They found it to be a reducing sugar which was unfermentable by yeast. It could not be oxidized by bromine water, thus indicating a ketone structure, a conclusion borne out by the fact that on reduction it yields two heptitols. It forms a crystalline phenylosazone which yields an osone in the usual way.

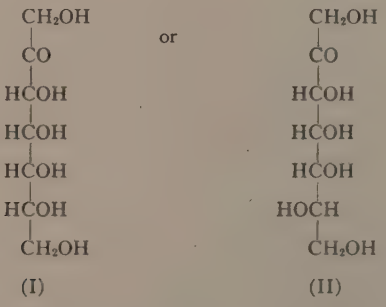
When the sugar is boiled with dilute acids it loses some 80% of its reducing power towards Fehling's solution and its optical rotation changes from a small positive to a large negative value. They ascribed this behavior to the

formation of anhydro-sugar by the loss of one molecule of water. La Forge and Hudson first isolated this anhydride by treating sedose with benzaldehyde in the presence of 70% sulphuric acid, when a dibenzal derivative corresponding to that of the anhydride of the heptose (as shown by analysis and molecular weight determinations) was formed. Decomposition of this dibenzal derivative with acetic acid yielded the anhydride as a syrup which, by solution in 95% alcohol, could be obtained in crystalline form (m. p. 155° C. and $[\alpha]_D^{20} = -146.3°$).

Sedosan (anhydro-sedoheptose) shows no mutarotation in aqueous solution and is non-reducing towards Fehling's solution. The latter fact may be taken as evidence that the oxygen atom of the carbonyl group of sedose participates in the anhydride formation. On treatment with dilute acids both the free sugar and the anhydride give an equilibrium mixture containing approximately 20% of the free sugar and 80% of the anhydride, as shown by rotation values and reducing power.

In a later communication (11) La Forge produces evidence to show that sedose has a straight- and not a branched-chain structure. One of the two heptitols formed on reduction is optically inactive while theoretically all those derived from branched-chain formulae must be active. Furthermore, oxidation of these alcohols by nitric acid gives rise to dibasic pentahydroxy pimelic acid only, and not to a tribasic acid as would be expected from a branched-chain alcohol.

From a consideration of the two heptitols derived from sedose, in relation to the known heptitols derived from the other sugars, La Forge (11) reached the conclusion that sedose must be an α-ketose having one of the following configurations:



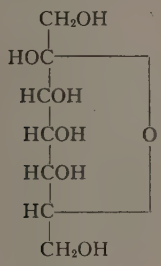
(I) (II)

either of which fulfils the necessary conditions of yielding one active and one inactive heptitol differing from the known ones.

No attempt was made by this author to determine the structure of the anhydride and it seemed that light might be thrown on the matter by an application of the method of degradative oxidation of methylated sugar derivatives developed by Haworth and coworkers.

The free sugar, sedose, may have either a furanose or a pyranose ring structure, although the former is unlikely in view of the stability of the sugar

and the findings of Haworth and his coworkers as to the ring structure of the normal sugars. Ignoring for the moment the position of the anhydride ring in sedosan, and assuming the free sugar to have the structure indicated by (I), it is seen that nitric acid oxidation of the fully methylated sedose IV will yield trimethoxy riboglutaric acid V, from the pyranose form (III), and inactive dimethoxy succinic acid VIII from the furanose type (VI).



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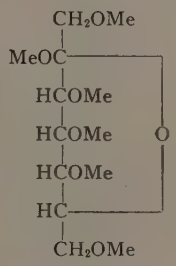

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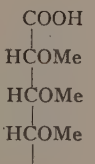

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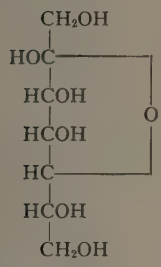

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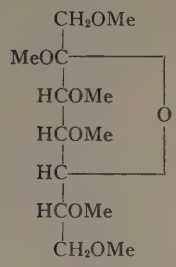

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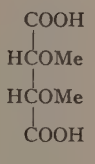
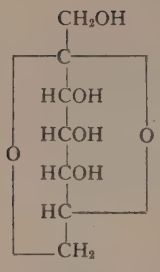


(VIII)

Hence one or other of these acids should be obtained irrespective of the position of the anhydride ring. The same products should also be obtained if the free sugar has the structure II.

In the case of sedosan the most probable positions for the anhydride ring appear to be 2:5 or 2:7 if the sugar ring is pyranose (III), and 2:6 or 2:7 if it is furanose (VI). This gives rise to the six possible formulae IX, X, XI, XII, XIII and XIV, when the two possible configurations I and II of the sugar are also considered. It may be noted that formula XII is unlikely since it would stand in contradiction to Hudson's rule in that sedosan has a negative and sedose a positive rotation.

Structures IX and X should give on methylation isomeric 1:3:4:5-tetramethyl sedosans XV and XVI, and XV on oxidation should yield a mixture of trimethoxy riboglutaric acid XXII and 2:3:4-trimethoxy-5-hydroxy-*d*-allomucic acid XXI, while XVI should give the former (XXII) together with 2:3:4-trimethoxy-5-hydroxy-*l*-talomucic acid XXIII. In a similar manner the



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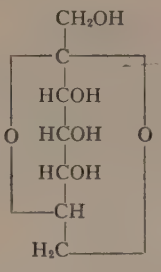

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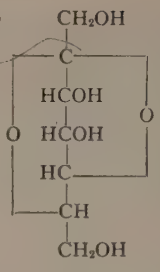

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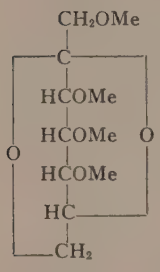

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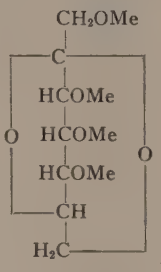

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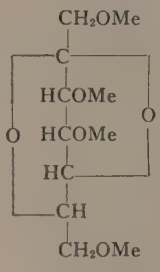

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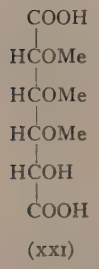

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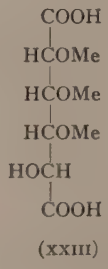

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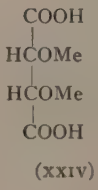
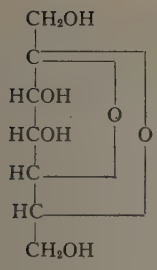


(XXIV)

(XXII)

methylated products XVII, XVIII, XIX and XX (obtained from the anhydro-sugars XI, XII, XIII and XIV respectively) should yield the following oxidation products:

The methylated products XVII and XVIII should both yield a mixture of inactive dimethoxy succinic acid XXIV and 2:3-dimethoxy-4-hydroxy-riboglutaric acid XXV; XIX should give a mixture of 2:3:5-trimethoxy-4-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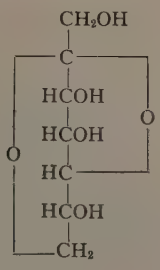
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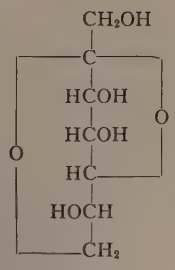
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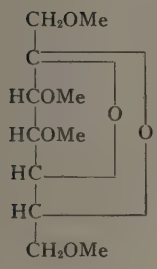
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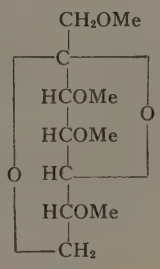
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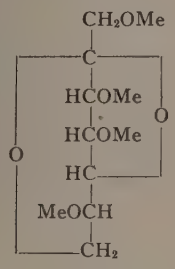
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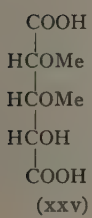
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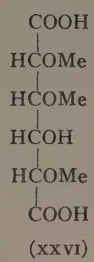
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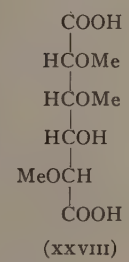
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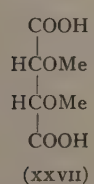
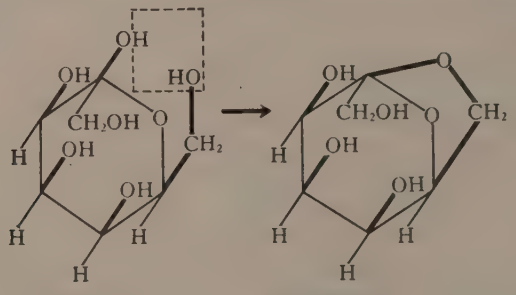

(XXVII)

hydroxy-riboglutaric acid XXVI and inactive dimethoxy succinic acid XXVII. The latter acid (XXVII) should also be obtained, together with 2:3:5-trimethoxy-4-hydroxy-*l*-talomucic acid (XXVIII) by the oxidation of of XX.

In order to decide which of the formulae IX to XIV is applicable to sedosan the compound was completely methylated by means of Purdie's reagents (using methyl alcohol as extraneous solvent during the preliminary operations) and a crystalline tetramethyl derivative obtained, m.p. 48-49° C.,

$[\alpha]_D^{20.5} = -137°$ (c = 1.136) in water. The product was non-reducing towards Fehling's solution and showed no mutarotation. The fully methylated sedosan was oxidized by treatment with 10 times its weight of nitric acid (d = 1.42) at 90° C. and the resulting acid esterified by refluxing with methyl alcohol containing 3% HCl. The esters were converted into the methylamides by the procedure of Haworth and Jones (2). From the mixture an inactive trimethoxy glutaric acid was isolated in the form of its crystalline dimethylamide, m.p. 145-146° C. but no trace of the diamide of inactive dimethoxy succinic acid could be isolated or detected, thus indicating absence of the free acid in the oxidation product. Accompanying the crystalline methylamide was a syrupy methylamide which could not be induced to crystallize. Analysis of the latter indicated that it was possibly dimethoxy hydroxyglutaro-methylamide, although too much reliance cannot be placed on the analytical figures owing to the syrupy nature of the product.

The presence of the trimethoxy glutaric acid in, and the absence of inactive dimethoxy succinic acid from the oxidation product is strong evidence of the ring system of sedosan being in the 2:6 and 2:7 positions (formulae IX and X). If such is the case sedosan itself would have but one primary alcohol group. Now Helferich has shown (4, 5, 6) that trityl chloride condenses only with primary alcohol groups in the sugars and their derivatives. Accordingly in order to test the above conclusion sedosan was condensed with an excess of trityl chloride under the conditions described by Helferich. The product isolated was found to have m.p. 147° C., $[\alpha]_D^{23.8} = -33.25°$ in chloroform (c = 1.199). Analysis showed it to be the monotrityl derivative. The yield was 75% theoretical. A small quantity of tritanol was also isolated.



Sedoheptose *Sedosan (Anhydro-sedoheptose)*

An attempt to prepare the methylamide of trimethyl δ-ribonolactone for the purpose of identifying definitely the crystalline methylamide (A) obtained from the inactive product was unsuccessful owing to the inability to effect the epimerisation of trimethyl δ-arabonolactone into the corresponding ribonolactone. It was found however that the amide (A) was not identical with arabo-trimethoxy-glutaromethylamide.

It thus appears highly probable that sedose is a normal pyranose sugar (III) and that sedosan has the anhydride ring joining the 2:7 carbon atoms (IX) or (X). That neither trimethoxy hydroxyallomucic nor 2:3:4-trimethoxy-5-hydroxy-talomucic acid was found among the oxidation products may be attributed to the opening of the anhydride ring by the nitric acid prior to oxidation, the point of attack thus being eliminated.

A decision between formulae IX and X can be arrived at by a comparison of the carbon models for each of the two configurations. It is seen that where all the OH groups in the free ketone form of sedoheptose are on the same side of the plane of carbon atoms (Formula I), the formation of a pyranose ring can readily take place. Furthermore, in this pyranose ring structure the spatial relations of the hydroxyl groups attached to carbon atoms (2) and (7) are very favorable for the removal of a mol of water, with formation of a furanose ring involving little or no strain in the bicyclic acetal structure of the resulting anhydro-sugar. On the other hand, if the (OH) group attached to carbon atom (6) were situated on the opposite side of the plane (II), the likelihood of formation of a pyranose ring from the free ketone sugar would seem, for spatial reasons, to be very remote.

Experimental

Methylation of Sedosan

Ten grams of pure sedosan*, crystallized from 95% alcohol and having m.p. 155° C. and $\left[\alpha\right]_D^{28} = -147.3°$ (c = 2.136) in water, was dissolved in 200 cc. of methyl alcohol and treated with 12 cc. of methyl iodide and 22 gm. of silver oxide, at 50° C. for 18 hr. On extraction with methyl alcohol and removal of the solvent a pale-yellow vitreous solid was obtained, methoxy content = 16.8%. The material was re-methylated four times with the same reagents but using no extraneous solvent during the two final operations. By this procedure 9.0 gm. of a fairly viscous, pale-yellow syrup was obtained which, on distillation under high vacuum, yielded the following fractions:

TABLE I

FRACTIONATION OF METHYLATED SEDOSAN

Fraction	Bath temp. in ° C.	B. P. in ° C.	Press. in mm.	Weight in gm.	n_D^{20}	Remarks
1	145–150	116	0.21	1.95	1.4683	Colorless
2	145–150	116	0.19	3.01	1.4682	Colorless
3	150	118	0.17	1.70	1.4691	Colorless
Residue in flask	—	—	—	1.27	—	Dark-brown

**This was supplied through the kindness of Dr. La Forge, to whom the authors' best thanks are due.*

Each of the fractions was devoid of reducing action towards Fehling's solution and all crystallized on standing: fractions 1 and 2 completely, fraction 3 almost completely. The material, when recrystallized from a mixture of

ether and light petroleum, separated in the form of small square plates, m.p. 48-49° C. Methoxy content = 49.5%, calculated for $C_{11}H_{20}O_6$ = 49.9%, $[\alpha]_D^{20.5} = -137°$ (c = 1.140) in water. There was no mutarotation shown in 48 hr.

Oxidation of Tetramethyl Sedosan

Three grams of crystalline tetramethyl sedosan was dissolved in 30 cc. of nitric acid (d = 1.42) and the temperature of the solution gradually raised. At 80-85° C. gas evolution became vigorous, and the solution was maintained at that temperature until the reaction had subsided (10 hr). The mixture was diluted considerably with water and concentrated to a syrup under diminished pressure at 40° C. Water was distilled continuously through the product in order to remove the nitric acid, following the method of Hirst and Purves (10). After removal of the nitric acid, 50 cc. of methyl alcohol was distilled through the mixture in order to eliminate water.

Esterification of the Oxidation Product

The syrup was dissolved in 60 cc. of methyl alcohol containing 3% of HCl and the solution heated under reflux for seven hours. The acid was neutralized by means of silver carbonate, and the filtered solution concentrated under diminished pressure at 50° C. to give a pale-yellow syrup which was distilled in high vacuum and fractions collected as shown in Table II.

TABLE II

FRACTIONATION OF THE ESTERIFIED OXIDATION PRODUCT

Fraction	Bath temp. in ° C.	Pressure in mm.	Weight in gm.	$n_D^{25.5}$	Remarks
1	130–140	0.07	0.56	1.4394	Pale-yellow
2	150–160	0.05	1.43	1.4593	Pale-yellow
3	165–180	0.07	0.46	1.4708	Yellow
Residue in flask	—	—	0.58	—	Dark-brown

The following methoxyl contents were found:

Fraction 1 = 55.2%; fraction 2 = 50.2%; fraction 3 = 47.0%.

(Theoretical value for dimethyl trimethoxyglutarate (x) = 62.0% OCH_3; dimethyl dimethoxy-hydroxyglutarate(y) = 52.5%; dimethoxy hydroxyglutarolactone = 45.6%.) Fraction 1 thus appeared to be a mixture of the trimethoxy glutarate and the dimethoxy hydroxyglutarate, and the same was true of fraction 2. Fraction 3 was a mixture of the latter ester and the corresponding lactone. These conclusions were borne out by the titration values:

0.154 gm. of fraction 1 required 12.7 cc. 0.1 *N*. NaOH (calculated for *x*: 12.3 cc., for *y*: 13.0 cc.).

0.401 gm. of fraction 2 required 33.4 cc. 0.1 *N*. NaOH (calculated for *x*: 32.1 cc., for *y*: 34.0 cc.).

No indication of the presence of inactive dimethoxy succinic acid was found.

Preparation of the Methylamides from the Esters

Fraction 1 was dissolved in 6 cc. of methyl alcohol and saturated at 0° C. with methylamine, prepared by treatment of methylamine hydrochloride with caustic soda. The mixture was allowed to stand at room temperature for two days, re-saturated with methylamine and allowed to stand for a further three days. The methyl alcohol was removed under reduced pressure, whereupon the product crystallized leaving a small quantity of syrup. The solid was recrystallized from a mixture of benzene and petroleum ether in the form of small granular crystals, m.p. 143-144° C. Recrystallization from benzene yielded short columnar needles, m.p. 145-146° C. The crystals had zero rotation in water. Analysis: Found: C = 48.12%, H = 8.2%, N = 11.5%, OMe = 37.5%; calcd. for $C_{10}H_{20}O_5N_2$ (trimethoxy riboglutaro-dimethylamide) C = 48.4%, H = 8.1%, N = 11.3%, OMe = 37.5%.

Fraction 2 was treated similarly with methylamine but yielded no solid product. The syrupy methylamide had $\left[\alpha\right]_D^{24} = -18.3°$ (c = 0.569) in chloroform. Analysis: Found: C = 44.1%, H = 7.7%, N = 11.5%, OCH_3 = 25.0%; calcd. for $C_9H_{18}O_5N_2$: (Dimethoxy hydroxyglutaro-methylamide) C = 46.1%, H = 7.7%, N = 12.0%, OCH_3 = 26.5%.

Preparation of Trimethoxy Araboglutaro-dimethylamide

Thirty grams of *l*-arabinose was dissolved in 70 cc. of water and methylated by means of dimethyl sulphate and caustic soda in the usual way. The product was re-treated with the same reagents and then subjected to three methylations by Purdie's method, after which the material had a methoxyl content of 59.9%.

It was distilled under high vacuum, and the fractions shown in Table III separated.

TABLE III

FRACTIONATION OF TRIMETHOXY ARABOGLUTARO-DIMETHYLAMIDE

Fraction	Bath temp. in ° C.	B. P. in ° C.	Pressure in mm.	n_D^{24}	Weight in gm.	Remarks
1	112	77	0.085	1.4462	4.96	Colorless
2	112–115	82–85	0.085	1.4483	8.48	Colorless
3	90–95	84	0.010	1.4488	9.90	Colorless
Residue in flask	—	—	—	—	0.30	Brown

On standing, each of the three fractions crystallized, m.p. 44-45° C., $\left[\alpha\right]_D^{25.4} = +71.7°$, in close agreement with the accepted constants of Drew and Haworth (1). The trimethyl methylarabinoside (7.2 gm.) thus obtained was hydrolized by 3% aqueous HBr, as recommended by Pryde, Hirst and Humphreys (13), at 85° C. for one hour, and this followed by oxidation with bromine water.

The product was worked up in the usual way and heated under diminished pressure at 90° C. for three hours in order to promote formation of trimethyl δ-arabonolactone.

Attempted Epimerisation of Trimethyl δ-Arabonolactone

The trimethyl δ-arabonolactone (7.0 gm.) obtained as above, was mixed with 70 cc. of water containing four grams of pyridine, and heated in a boiling water-bath for 105 hr. in order to effect epimerisation according to the method of Haworth and Long (3). The solution was considerably diluted with water and this removed under diminished pressure with continuous addition of more water until no further smell of pyridine could be detected in the distillate.

The resulting syrup (4.0 gm.) was oxidized by means of 40 cc. of nitric acid (d = 1.42). Vigorous oxidation commenced at 75° C., the mixture was cooled to 60° C. and the reaction allowed to proceed for eight hours. The product was isolated in the manner previously described and esterified in 80 cc. of methyl alcohol containing 3% HCl. The resulting ester was dissolved in 40 cc. of dry methyl alcohol, saturated with methylamine and the mixture allowed to stand for 48 hr. at room temperature. On removal of the methyl alcohol short colorless needles of m. p. 167° C. were obtained. Recrystallization from a mixture of methyl alcohol and ether gave silky needles of m.p. 171.5° C., $[\alpha]_D^{24.5} = +63.8°$. (Haworth and Jones (2) record m.p. 172° C. and $[\alpha]_D^{18} = +59.9°$ for the dimethylamide of trimethoxy araboglutaric acid obtained by them.)

It thus appears that the epimerisation to trimethyl δ-ribonolactone acid took place to a very small degree, as none of the corresponding trimethoxy ribo-glutaro-methylamide could be isolated. Admixture of the above trimethoxy araboglutaro-methylamide with the methylamide from the tetramethyl sedosan oxidation gave m.p. 130° C. showing strong depression.

Preparation of the Trityl Derivative of Sedosan

One gram of sedosan (one molecular proportion) was treated with 2.6 gm. (two molecular proportions) of trityl chloride in 8 cc. of pyridine for one hour on the boiling water-bath. After standing overnight the solution was poured into ice water, giving a gummy material which hardened to an amorphous powder on rubbing under water, the latter being frequently changed. The dried product (3.8 gm. m.p. 98° C.) was dissolved in benzene, and petroleum ether added until a turbidity was formed. The granular mass which separated from the solution was recrystallized from chloroform, when crystals, m.p. 149-150° C. separated. (Recrystallization gave triphenyl carbinol, m.p. 159° C. The rotation was almost zero, due to slight impurity.) The mother liquor yielded a crystalline product, m.p. 146-147° C. which was raised to 147-148°C. on further recrystallization. $[\alpha]_D^{24} = -33.25°$, (c = 1.199) in chloroform. Analysis: Found: C = 71.24%, H = 6.50%; calcd. for $C_{26}H_{26}O_6$ (Mono-). C = 71.89%, H = 6.00; for $C_{45}H_{40}O_6$ (Di-.). C = 79.88, H = 5.92. Trityl group (Helferich and Sieber's method (6)), found: 55.7%; calcd.: 56% (mono-), 70% (di-).

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DETERMINATION OF CARBON AND HYDROGEN[1]

By Edgar Stansfield[2] and John W. Sutherland[3]

Abstract

This paper describes modifications of the ordinary combustion apparatus for the determination of carbon and hydrogen in organic matter. These modifications were developed for analysis of coal, but are suited to more general use. It is claimed that they save time, and reduce the liability to error so that less practice and skill are required to obtain consistently good results. The combustion tube can be readily emptied, cleaned and repacked; with reasonable care it has a long life.

In the Liebig system the combustion tube is packed with copper oxide and with lead chromate in bulk, these reaction materials being held in position and separated from each other by plugs of oxidized copper gauze. A removable diffusion spiral of oxidized copper gauze is also inserted in the inlet end of the tube. In the Dennstedt system the copper oxide is replaced by a platinum contact star, the lead chromate by a mixture of lead peroxide and minium placed in two porcelain boats, and no diffusion spiral is required.

The Liebig tube provides ample facility for complete combustion, but the tube is always destroyed, more or less rapidly in the heated zone, by the copper oxide packing. The lead chromate may also cause damage. The diffusion spiral cannot be heated rapidly at the beginning of the determination, and the heating of this spiral is apt to cause undue heating of the stopper at the inlet end. The Dennstedt system is rather more complicated, but no harmful reaction material touches the heated tube. On the other hand, if at any time there is at all a rapid evolution of volatile matter from the coal, the oxygen supply is held back when most needed, and incomplete combustion is apt to result. In this system there is no reserve of "solid" oxygen, as when copper oxide is present.

In the system to be described, complete combustion is ensured by use of both copper oxide and contact platinum. The tube is protected by placing the copper oxide and lead chromate in porcelain boats. An internally heated quartz tube is used instead of the copper oxide diffusion spiral. Precautions are provided to prevent, on the one hand, condensation of moisture at the outlet end of the tube, or on the other hand, overheating and decomposition of the rubber sleeve or stopper at either end of the tube. Any ordinary combustion furnace, purifying train, and absorption train can be used.

In the apparatus employed in this laboratory a multiple unit electric furnace is used. The trough for the tube is 35 in. long. There are three movable heaters, 5 in., 13 in. and 9 in. over-all length, respectively, and each is provided with its own rheostat. A thermocouple is permanently installed in each of the two longer heaters to permit accurate temperature control.

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Contribution from the laboratories of the National Research Council of Alberta, University of Alberta.

[2] *Chief chemical engineer, Research Council of Alberta.*
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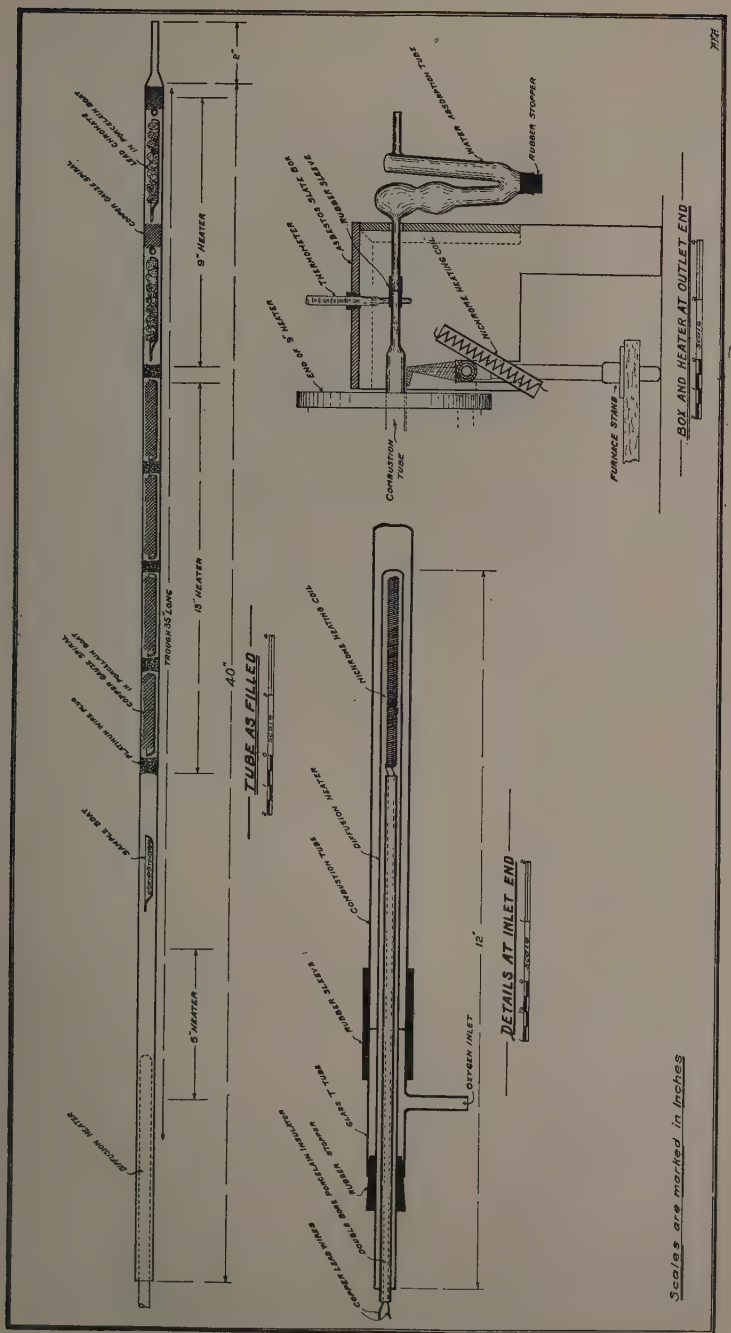

FIG. 1. *Combustion tube and accessories.*

The tube employed is made of clear quartz, 40 in. long, with a bore of 9/16 in. (14 mm.). The exit end is reduced and fused to a quartz tube, 2 in. long and 1/8 in. (3 mm.) bore. The total length is thus 42 in. or 7 in. longer than the trough of the furnace. The tube is packed as follows, commencing at the reduced or exit end: in the portion of the full bore tube projecting from the furnace, a copper gauze spiral about 3/4 in. long; inside the end heater, 9 in. long, two or three porcelain boats, filled with small lumps of fused lead chromate, and separated from each other by small plugs of oxidized copper spiral; inside the middle heater, 13 in. long, four porcelain boats each filled with an oxidized copper gauze spiral and alternating with five plugs of fine platinum wire. The end and middle heaters are placed about a half-inch apart to allow of entrance of the thermocouple wires. This packing of the tube leaves a length of about 17 in. clear at the inlet end for the boat containing the sample and for the diffusion heater.

The gauze spiral plugs are about 3/4 in. long, just fill the bore of the tube, and have a loop for ease in removal. The platinum plugs are loosely made of scraps of hair wire, 0.004 in. diameter (from bomb calorimeter use). The porcelain boats are No. 6 Coor's combustion boats (88 mm. × 12 mm.) with the handle end cut off to economize length and to encourage passage of gas over the reaction material. A small hole is ground in the other end of the boat. The boats are filled with oxidized copper gauze spirals, or with lead chromate, just not to touch the top of the tube. The boats can be cut with nichrome wire moistened with a mixture of carborundum and oil.

The diffusion heater consists of a quartz tube 12 in. long by 3/8 in. outside diameter, closed at one end. A small nichrome wire heater, three inches long, wound round a small quartz tube, is placed in the closed end of the larger tube. Electrical connections are made with copper wires inside alundum insulating tubes. This heater when in use projects seven or eight inches into the combustion tube and therefore does not overheat the connection at the inlet end, even when the closed end of the heater is red hot. The temperature is controlled with a rheostat.

The inlet end of the combustion tube is permanently connected by a rubber sleeve to one end of a short T-tube, of the same outside diameter. The other end of the tee is closed by a rubber stopper carrying the diffusion heater. This stopper is removed to permit the insertion of the sample boat. The side tube of the tee is of small bore and is permanently connected to the purifying train.

The reduced exit end of the tube is enclosed in a small box made of asbestos slate held together with brass angle strips. The air inside this box is maintained at a temperature of about 70° C. by means of a small nichrome No. 16 gauge wire coil connected in series with the end heater. A thermometer is inserted in the top of the box with its bulb touching the rubber sleeve connection. The temperature of 70° C. is high enough to prevent condensation of moisture in the connecting tubes, and yet causes no damage to the rubber sleeve. The suitable size of heating coil was found by trial, and final adjust-

ment of temperature made by raising and lowering the coil until the correct position was determined.

Normal purification and absorption trains are used as follows: Purification—sulphuric acid, potash (50% solution), soda lime and calcium chloride, all in duplicate for air and oxygen respectively, followed by a control stopcock and one small tube of dehydrite (magnesium perchlorate trihydrate); absorption—dehydrite in special U-tube, potash (50% solution), and dehydrite in Vanier bulb, soda lime and dehydrite in check U-tube. These are followed by a calcium chloride or dehydrite guard tube, and a Marriott bottle arranged to give just sufficient suction to draw gas through the Vanier bulb. The control stopcock should be of small bore. Accuracy of control is increased by filing a small groove across each end of the hole through the plug.

Few points need special mention with regard to manipulation. Before commencing the determination the length of the tube packed with lead chromate, is heated to 400° C. and the copper oxide and platinum length to 800° C., whilst a slow current of oxygen is passed through. The entrance end of the tube is left cold. The weighed absorption train and Marriott bottle are attached to the exit end. The stopper and heater are removed from the entrance end, the heater being laid down on two wire supports, and the boat with the weighed sample of coal inserted. This boat is placed about three inches from the first platinum plug. The diffusion heater and stopper are replaced and the current turned on to this heater. One minute is sufficient to bring this to a red heat. The oxygen current should not be turned off whilst the boat is being inserted, but after the stopper is replaced as above, the oxygen is cut off momentarily whilst the exit tap to the Marriott bottle is slowly turned full on. The oxygen supply is then regulated by the control stopcock.

The regular tube heater can be used to heat the sample boat as soon as the oxygen current is regulated, because of the quick heating of the diffusion heater. However, with unstable, high-moisture coals, the oxygen heated by passing over the diffusion heater is itself sufficient to dry the sample, and may even start decomposition. Combustion with any sample is completed as usual; after which it has been found that 1.25 litres of air are sufficient to displace oxygen completely from the apparatus.

The units of the absorption train are weighed before and after the determination against dummy units of the same type employed as counterpoises. They should always be dusted with a camel-hair brush before they are set aside to cool in the balance case. In very dry weather these tubes are apt to become electrified when dusted, and their apparent weight is thus changed. This electricity can be discharged by keeping some radioactive mineral in the balance case, or by breathing on the tubes before they are set aside. This last precaution is not necessary with the Vanier bulb as it is safer to leave this at least half an hour to cool.

The foregoing apparatus and devices have been evolved through many years of experience, beginning in Ottawa and continued in Edmonton. Many chemists have assisted and it would be impossible to attempt to give due credit for the final form.

NOTE ON THE COMBINED USE OF PHOTO-ELECTRIC CELL AND PROJECTION MICROSCOPE[1]

By Alfred Savage[2] and M. C. Jamieson[3]

Abstract

A method is described whereby the comparative areas of irregularly shaped microscopic objects may be rapidly determined. It consists of staining them with fuchsine and projecting their magnified images into a photo-electric cell insensitive to red light. The photo-electric current is inversely proportional to the sizes of the images and, at low magnifications, may be measured with a sensitive galvanometer.

Studies of variation in the sizes of certain cells have been reported by numerous observers in different fields of biology. Comparatively recent examples include the publications of Price-Jones (6) on human red blood cells, of Savage, Williams and Fowler (7) on the spermatozoa of bovines and of Levine (2, 3), Bailey (1) and Newton and Johnson (5) on the spores of the wheat rust fungus. All of these studies were based on linear measurements often made in one dimension only.

In dealing with cells of reasonably uniform shape, measurements taken in one dimension may serve as a basis for estimating their comparative sizes but, when irregular forms are concerned, such as pathological sperm or the blood cells found in some types of anaemia, it is obvious that simple linear measurements fail to afford such a criterion. The apparent areas of such cells may be obtained through application of a method described by Scammon and Scott (8) and by Mainland (4). This technique involves so much time and labor, however, that it is questionable if those interested in biomicrometry will apply it extensively. Something more rapid is required.

Though confined to preliminary observations, this note indicates that a more rapid means for obtaining the comparative areas of isolated cells is now available. It consists of the combined use of a projection microscope, a red dye and a photo-electric cell insensitive to red light. These will be described briefly and in reverse order.

Photo-electric Cell

The writers are acutely aware that while the principle upon which the photo-electric cell depends and the uses to which it is commonly put are familiar to physicists and astronomers, most biologists know little or nothing about it. For this reason it is suggested that texts dealing with the subject be consulted for details. At present it will suffice to say that the apparatus is a device which permits current to flow through it when exposed to the action of light.

[1] *Manuscript received August 6, 1930.*

Contribution from the Manitoba Agricultural College, Winnipeg, Canada, with financial assistance from the National Research Council.

[2] *Professor of Animal Pathology, Manitoba Agricultural College.*

[3] *Lecturer in Bacteriology.*

It consists of a highly evacuated glass bulb in which are sealed the photo-sensitive material and the anode, both connected externally to suitable terminals. Its action differs from that of the selenium cell and other devices having the same general action in at least two important features. First, it is highly sensitive and, second, its output is directly proportional to the quantity of incident light. The response to light of different colors is largely a matter of the metal used as cathode, and cells possessing a considerable variety in this respect are now available. Because proportionally little sensitivity to red light was desired for the work in hand, a sodium or potassium cathode seemed imperative. For this as well as other reasons the particular type selected was known as a Burt Standard UV-Na.2 cell arranged for side exposure.

This was mounted upright in a bakelite socket screwed to a five-inch circular plate of the same material. The plate was provided with legs by attaching three rubber stoppers to the under side with Canada balsam.

A box constructed of wood and cardboard housed this tripod. Two holes were made in it. One was circular, one inch in diameter and directly opposite the cathode. This aperture was covered with a suitable piece of ground glass and served to admit light to the cell. The other opening was on the opposite side of the box and near the bottom. A small bakelite plate (2 in. × 3 in.) was cemented in place over it and served to support three binding posts through which the necessary electrical connections were made.

The cardboard box was then placed inside a slightly larger one of sheet copper, having similar openings, and the copper was electrically grounded to an iron water-pipe by means of a large wire soldered to both.

Red Dyes

The reason for using red dyes was exceedingly simple. The cell responds but slightly to wave-lengths of light between 6,000 and 7,000 Å. Consequently, when the image of an object, stained with fuchsine for example, was projected on to the window of the box, it registered virtually as black, as far as the response of the cell indicated. The size of the image being smaller than that of the window, and the remainder of the field being white, the electrical output was mainly caused by the difference between the two areas. If two or more objects were projected in succession and the amount of response noted in each case, the resulting figures afforded a means of comparing the areas of such objects providing they stained with equal intensity. On the other hand, if their affinities for the dye were unequal, this too would be shown even though the areas were the same. Thus each reading indicated the product of two factors, *i.e.*, area of image and staining intensity.

Projection Microscope

The word microscope is here qualified, not to designate a special type of instrument so much as to indicate the manner in which the instrument was employed. Any microscope so arranged with reference to an illuminant that it throws a magnified image upon some form of screen becomes a projection

apparatus for the time being. The instrument used conformed to the qualification only in this sense.

The stand was a substantial and complete English model, which remained steady at any inclination. It was used in a horizontal position. To obtain the largest amount of illumination, an apochromatic oil-immersion lens of the highest aperture ordinarily available was employed. The substage condenser was achromatic and was also immersed. Treated in this way its numerical aperture became 1.40.

An incandescent lamp with a single, concentrated metal filament served as light source. It was designed to operate at 54 watts. By means of a rheostat it was made to consume nearly twice that amount of energy. The result was brilliant and as satisfactory as anything previously obtained by the authors with the alternating current at their disposal. A suitably ventilated housing covered this bulb and permitted very little light to escape except through the one-half inch circular aperture intended for the purpose. In use, the lamp was placed about six inches from the substage condenser of the microscope.

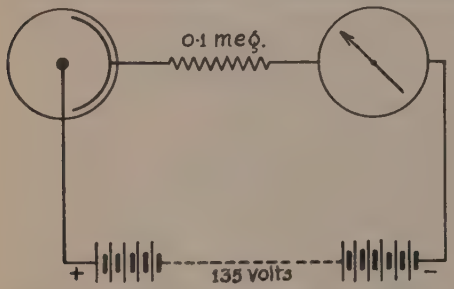

FIG. 1. *Diagram showing the connections of the photo-electric cell and the galvanometer.*

Other Apparatus

A d'Arsonval galvanometer sensitive to 0.00004 microampere and connected as shown in Fig. 1 was used. A potential of 135 volts was obtained by connecting three radio "B" batteries in series. The lamp and the galvanometer deflection scale were placed about 39 in. from that instrument.

Method of Use

The axis of the microscope, the source of light and the window leading to the photo-electric cell were brought carefully into optical alignment before any observations were attempted. Then the batteries were connected, the galvanometer adjusted and the overhead lights of the dark room shut off.

Observations

Red Rust Spores

The Dominion Rust Research Laboratory kindly supplied a generous lot of the uredospores of *Puccinia graminis* (*tritici*). These were stained by boiling in carbol-fuchsine for 10 min. They were then centrifuged and the stain decanted. Repeated washing with water and centrifuging followed until the wash water was colorless. The spores were then dried and some of them mounted in glycerine jelly between an ordinary microscope slide and coverslip.

At 500 diameters magnification images of these were projected into the photo-electric cell; the galvanometer reading of a blank field was then noted prior to the deflection caused by each spore as well as afterwards. A series of 500 readings gave the following frequency distribution.

TABLE I

SIZES AND FREQUENCY DISTRIBUTION OF RUST SPORES AT 500 DIAMETERS AS INDICATED BY THE PHOTO-ELECTRIC CELL

Deflection of galvanometer in mm.	2	3	4	5	6	7	8	9	10	11
Frequency	1	10	37	77	112	115	93	42	12	1

The lengths of the images of an equal number of spores taken at random, as projected on a small white screen at the same magnification, were measured. The frequency with which the various lengths occurred is shown in Table II.

TABLE II

LENGTHS AND FREQUENCY DISTRIBUTION OF RUST SPORES*

Length, in mm.	11	12	13	14	15	16	17	18	19
Frequency	4	16	55	137	141	91	38	13	5

**At 500 diameters.*

As expected, a normal curve resulted in each case. The amount of variation however is greater in the first instance than in the second. Evidently all spores of a given length do not have the same diameter and staining intensity. This point is shown graphically in Fig. 2.

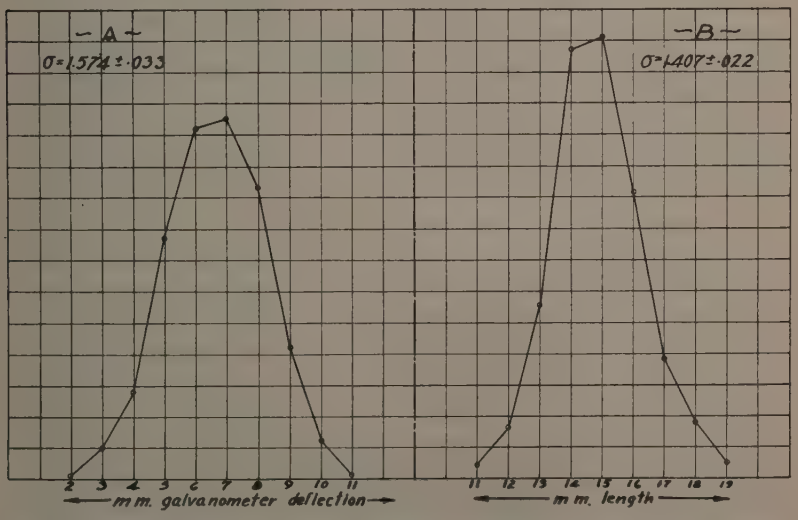

FIG. 2. *Frequency distribution curves obtained by measuring 500 uredospores of Puccinia graminis. In A the class intervals represent areas as seen by the photo-electric cell; in B the intervals indicate millimetres of length. Magnification ×500 in both cases.*

Red Blood Cells

The same procedure was attempted in the case of a film of normal blood but it was found that, at about 1,200 diameters magnification, the amount of light obtainable through the microscope was not sufficient to cause a galvanometer deflection of consequence. The photo-electric current was therefore amplified by means of a thermionic valve.

It was an easy matter to obtain an immense amplification in this way, but, so far it has been found from experience that, under conditions used, accurate galvanometer readings are virtually impossible even when the instrument is not highly sensitive and the entire apparatus is electrically shielded. Whether or not this is because of the proximity of an electric railway line and a powerful radio broadcasting station has not been settled definitely.

Conclusion

In spite of technical difficulties of a purely electrical nature, it is considered evident that the application of a photo-electric cell to the microscope offers interesting possibilities and promises much.

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A STUDY OF THE HEAD LENGTH VARIABILITY OF EQUINE SPERMATOZOA[1]

By A. Savage,[2] W. L. Williams[3] and N. M. Fowler[4]

Abstract

The head lengths of horse spermatozoa have been measured in 51 samples and the measurements plotted in frequency distribution curves. These curves are unimodal and their coefficients of variation range from 4.00 to 6.00 (or slightly less). A coefficient in excess of 6.00 indicates an unsound sire, and animals from which skew curves are obtained may be regarded with extreme suspicion since they are not obtained in the case of normal animals. Generally the findings are in harmony with cytological data available, but both require further study before they can be reduced to a systematic basis.

Introduction

In 1927 Savage, W. W. Williams and Fowler (3) published "A statistical study of the head length variability of bovine spermatozoa and its application to the determination of fertility." The principal conclusions drawn by these writers may be summarized thus: (a) frequency distribution curves representing the head lengths of bovine sperm obtained from individual animals are usually in the form of normal curves; (b) in the case of healthy animals the curves have definite limits to their simple mathematical functions; (c) bulls whose sperm give skew curves or curves with functions exceeding the determined limits are invariably unsatisfactory as sires; (d) in certain cases this method of examination affords the only laboratory means by which such animals can be detected.

These findings have not been confirmed by other workers and, so far as the present authors are aware, no similar study has been made of the sperm of other domestic animals. Aside from any academic interest in the subject, it occurred to the authors that the practical value of these conclusions warranted the application of the same method to the study of horse sperm. Thanks to the favorable circumstances in which one of them (W) was placed this has now been done in the case of 47 stallions, from which 51 samples were obtained.

Scope

The present paper is therefore concerned with head length measurements of the spermatozoa of horses, the technique employed being identical with that of the above writers. Unfortunately, because of the comparatively small number of animals with which it has been possible to deal and the practical impossibility of learning, more than approximately, the actual reproductive performance of many high class stallions, it has not been possible to draw other than tentative conclusions.

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Previous Work

So far as could be ascertained, Wodsedalek (7) is the only previous worker in this particular field. Working with sections of testis at 2,000 diameters magnification, and selecting only mature sperms which were free in the lumens of the tubules and parallel to the objective, he measured the head lengths of 600 of them. Plotted in the form of a frequency distribution graph, his results showed a marked dimorphism, nearly equal numbers of the smaller and larger sperms varying about means of 4.75 μ and 5.5 μ respectively. He assumed that this difference in size was due to the presence of the accessory chromosome in 50% of the population. His observations were primarily cytological and not concerned with the fertility of the animal studied.

Material

The majority of the samples of semen with which the work was done were collected by one of the authors (W.L.W.) during the course of two seasons in a district where the breeding of race horses has long been an outstanding feature. With two exceptions they come from thoroughbreds. The contributing stallions represent a wide variety of reputations both as sires and as runners. For obvious reasons, their identities remain anonymous.

Technique

Preparation of Slides

Since full details with regard to the collection of semen and the preparation, fixation, "clearing" and staining of microscopic films have been reported elsewhere on several occasions (4, 5, 6), they need not be repeated here. Suffice to say that the technique followed was that employed by Savage, Williams and Fowler (3) in dealing with material from bulls.

Projection: Magnification

The procedure of these workers, really that of Parkes (2), was also adopted for the projection and measurement of microscopic images. Indeed, with two minor exceptions, the same apparatus was used. The first change consisted in keeping the optical system of the projection microscope uniform throughout the series of observations. This comprised a 3 mm. (60×) Zeiss apochromatic objective, 1.40 N.A., a 20× (K. 18) compensating eyepiece by the same maker, and an achromatic, oil-immersion substage condenser, 1.40 N.A., manufactured by Swift. At a projection distance of 62.5 cm. the magnification was 3,000 diameters.

The illuminant was also improved upon somewhat by using a concentrated, single filament lamp instead of the automobile head-light previously employed. The lamp referred to, as made by the General Electric Co., is known under the designation—"5.2-volt, 54-watt, standard base, T.10C. single filament lamp." By means of a specially constructed resistance coil (coupled in series) this lamp was operated from the usual electrical "house circuit" of 110 volts alternating current. After giving it a preliminary warming at five volts, the

pressure was gradually increased to 8.5 volts at which a flow of 12.5 amperes was registered. The result was splendid, and, even under such an excess load, the longevity of the individual lamps was remarkable.

Method of Work

This too has already been reported (3). In the case of each slide the length of the enlarged images of 500 sperm heads was measured to the nearest 0.5 mm. and the measurements afterwards plotted in the form of frequency distribution curves. The functions of the curves were then determined by the short method of Babcock and Clausen (1). In the computation of these functions the length of the sperm heads was not reduced to *microns*, but expressed in millimetres as on the projection screen.

All details concerning the identities and reproductive performances of the horses were unknown to those engaged in this work while it was in progress.

A technical difficulty was presented in the measurement of horse sperms under high magnification because it was often impossible to get both ends of a given head in focus simultaneously. This necessitated focussing many images twice and made progress very tedious and slow.

Accuracy

Two of the authors did all the microscopic work and the experience of having previously collaborated in the measurement of more than 50,000 bull sperms (3) gave good reason for believing that the factors of personal error were negligible. But, because the actual head lengths of horse sperms are only about two-thirds those of bulls, the proportional error of all the present measurements must be correspondingly greater. This, however, was unavoidable.

Classification of Stallions

It was a practical impossibility to ascertain the breeding records of the stallions and of the mares to which they were mated with any reasonable degree of accuracy. Thoroughbred breeders, as a class, apparently resent being questioned on this point. Many of them do not possess the information sought; others refused to give it.

In the area where almost all the stallions under consideration were kept, a group of men, comprising the owners of most of them, demanded a certificate of genital soundness, by a designated veterinarian, for each mare not owned by the proprietor of the stallion to which it was desired to breed her. In turn, the stallion owner offered as assurance, a certificate of genital health for his stallion, by the same veterinarian. But neither the certificates nor the examinations which preceded them eliminated genital inefficiency, the result being that, while some stallions were notably successful at procreation, others were notorious failures. The authors are certain only of these two extreme types. For the rest, it was considered that an attempted classification based on incomplete knowledge was bound to lead to error, so that it was not attempted.

TABLE I

FREQUENCY OF SPERM HEADS FOUND FOR EACH LENGTH × 3,000 IN MM.

Horse No.	13		14		15		16		17		18		19		20		21		22		23		24
1			1	0	1	3	1	5	12	20	37	73	126	96	61	44	15	3	1	1			
2							2	1	1	17	44	75	113	120	60	32	16	8	1				
3			2	3	2	3	7	20	30	47	84	95	71	66	46	17	2	1	4				
4			1	1	1	2	6	14	27	60	72	96	89	77	31	13	6	3	1				
5							4	6	8	23	37	57	70	106	103	49	27	8	2				
6				2	1	3	5	11	49	81	110	107	86	30	12	2	1						
7						1	2	6	18	46	78	135	105	63	32	10	3	1					
8				2	8	16	22	64	97	105	95	61	17	9	3	1							
9						1	1	3	4	11	16	46	69	88	101	75	51	19	8	6	1		
10								2	2	9	25	38	91	103	108	72	34	13	2	1			
11					2	0	1	7	12	41	95	133	105	59	33	7	4	1					
12	2	3	3	10	18	31	51	78	103	97	64	23	13	3	1								
13			1	0	2	3	16	37	53	93	106	81	58	31	14	4	1						
14		1	3	3	5	15	24	49	70	81	104	63	48	19	13	1	1						
15					1	3	19	37	67	104	104	91	51	19	4								
16			1	1	3	9	19	47	88	100	107	66	34	9	12	4							
17					1	6	10	21	57	97	134	83	66	18	7								
18					1	1	6	14	24	80	112	120	79	40	19	3	1						
19						1	10	18	47	88	141	107	61	19	4	3	1						
20							1	1	1	3	10	42	76	96	118	88	38	20	5	1			
21						1	1	3	4	20	33	71	95	87	82	52	30	11	9	1			
22								1	3	8	15	19	64	68	91	101	69	43	11	5	2		
23				1	3	3	4	22	46	65	82	82	76	56	33	12	6	4	5				
24				1	2	0	12	6	6	10	36	67	87	93	95	51	24	7	2	1			
25							2	11	9	20	62	100	135	100	40	13	5	2	0	1			
26				1	1	2	2	11	36	50	87	115	97	52	17	18	7	2	2				
27					1	3	8	23	51	73	116	96	74	35	12	5	2	1					
28							2	1	6	9	30	54	86	109	111	45	22	16	7	1	1		
29				1	2	2	7	18	37	76	113	100	76	40	22	5	1						
30					1	5	23	39	90	114	126	66	27	8	1								
31						1	0	1	9	26	54	110	134	95	49	16	3	1	1				
32	1	2	2	2	5	14	20	32	53	51	95	88	63	42	20	9	1						
33			1	1	2	4	7	8	24	31	59	96	103	67	66	22	6	3					
34			2	4	11	21	38	45	76	105	87	60	31	10	6	4							
35					2	3	8	15	42	56	114	106	92	45	15	1	1						
36								1	2	2	19	38	72	111	124	74	41	12	2	2			
37			1	1	2	3	6	14	23	46	102	99	99	57	28	10	4	5					
38				1	8	10	23	48	71	98	84	72	53	20	7	4	1						
39						1	3	2	7	22	37	69	94	97	100	40	16	10	1	1			
40							1	2	7	24	47	78	109	107	75	27	15	5	2	1			
41							3	2	14	23	60	89	113	110	56	19	8	1	2				
42				1	2	0	4	10	14	51	77	123	126	65	21	4	1	1					
43								8	34	55	115	114	100	49	19	4	1	0	1				
44						2	0	3	13	15	63	95	109	103	56	24	8	8	1				
45				2	0	1	0	6	11	37	84	95	121	74	42	17	5	1	3	0	0	0	1
46				2	4	2	16	25	51	73	110	96	61	35	16	7	2						
47							1	5	3	13	38	71	109	122	90	29	17	2					
48	2	0	0	4	6	10	18	27	56	75	82	94	56	28	19	7	11	2	2	1			
49						5	11	16	48	83	94	111	72	35	17	5	1	1	1				
50	1	2	0	2	0	2	2	5	12	34	80	96	96	85	57	12	8	4	2				
51			4	14	16	25	55	65	94	82	60	36	26	9	12	1	1						

TABLE II

SIMPLE FUNCTIONS OF THE CURVES OBTAINED FROM SPERM HEAD LENGTH MEASUREMENTS OF 51 SAMPLES OF HORSE SEMEN

No.	Mean head length	Standard deviation	Coefficient of variation
1	19.113±0.031	1.042±0.022	5.546±0.116
2	19.667±0.028	0.950±0.020	4.833±0.102
3	18.496±0.036	1.204±0.025	6.510±0.138
4	18.548±0.032	1.091±0.023	5.883±0.125
5	19.333±0.032	1.066±0.022	5.516±0.117
6	18.166±0.026	0.896±0.019	4.937±0.105
7	18.624±0.025	0.863±0.018	4.636±0.098
8	17.418±0.028	0.951±0.020	5.465±0.116
9	19.756±0.032	1.093±0.023	5.534±0.117
10	19.622±0.027	0.922±0.019	4.698±0.100
11	18.616±0.025	0.854±0.018	4.591±0.097
12	16.917±0.032	1.083±0.023	6.403±0.136
13	17.963±0.030	1.013±0.021	5.641±0.120
14	17.656±0.034	1.144±0.024	6.484±0.138
15	17.820±0.026	0.890±0.019	4.995±0.106
16	17.658±0.029	0.991±0.021	5.614±0.119
17	17.947±0.025	0.858±0.018	4.785±0.101
18	18.290±0.026	0.866±0.018	4.737±0.100
19	18.042±0.024	0.811±0.017	4.496±0.095
20	19.799±0.026	0.896±0.019	4.526±0.096
21	19.402±0.032	1.071±0.022	5.520±0.117
22	20.071±0.031	1.053±0.022	5.247±0.111
23	18.419±0.035	1.192±0.025	6.471±0.137
24	19.266±0.034	1.152±0.024	5.984±0.127
25	18.869±0.026	0.877±0.018	4.651±0.099
26	18.509±0.030	1.027±0.021	5.533±0.118
27	18.144±0.028	0.955±0.020	5.266±0.112
28	19.509±0.030	1.001±0.021	5.132±0.109
29	18.230±0.028	0.959±0.020	5.261±0.122
30	17.614±0.024	0.811±0.017	4.608±0.098
31	18.920±0.024	0.809±0.017	4.276±0.091
32	17.980±0.037	1.262±0.026	7.020±0.149
33	18.756±0.033	1.125±0.023	6.000±0.127
34	17.437±0.033	1.118±0.028	6.415±0.136
35	18.252±0.027	0.918±0.019	5.032±0.107
36	19.736±0.026	0.867±0.018	4.394±0.093
37	18.489±0.031	1.057±0.022	5.719±0.022
38	17.711±0.032	1.075±0.022	6.073±0.129
39	19.272±0.030	1.008±0.021	5.232±0.111
40	19.154±0.028	0.940±0.020	4.903±0.104
41	18.966±0.027	0.907±0.019	4.782±0.101
42	18.543±0.026	0.873±0.018	4.709±0.100
43	18.424±0.024	0.825±0.017	4.479±0.095
44	19.018±0.028	0.940±0.020	4.945±0.105
45	18.789±0.029	0.979±0.020	5.214±0.111
46	18.072±0.031	1.048±0.022	5.800±0.123
47	19.238±0.026	0.866±0.018	4.502±0.095
48	18.032±0.039	1.311±0.027	7.275±0.154
49	18.178±0.029	0.975±0.027	5.36 ±0.114
50	18.790±0.032	1.095±0.023	5.83 ±0.124
51	17.138±0.037	1.235±0.026	7.20 ±0.153

Observations

Sperm head length measurements of 51 samples of semen gave the figures shown in Table I. (One horse provided three samples in the course of two

seasons; two others were duplicated in a single year.) The simple functions of these curves are set forth in Table II. It is to be noted that the coefficients of variation range from 4.726 (±0.0910) to 7.275 (±0.1549).

Discussion

Limits of Variability

Fig. 1 shows the coefficients of variation arranged, according to their frequencies, into classes beginning at 4.00 and progressing therefrom by intervals of 0.333. There are two reasons for arranging them in this way. In the first place, it is a biometric convention that, in dealing with a series of this kind, differences between any two classes may be considered as mathematically significant if they be as great as, or greater than, thrice the average probable error. Secondly, this arrangement is very convenient. Moreover, having arranged the coefficients in several other ways, this one was found to be the most convincing.

It is clearly indicated in Fig. 1, that the coefficients fall into two main groups, that to the left of the class 6.00-6.33 and that to the right of it. The latter group is of special interest because it includes all the animals which were known to be definitely poor and, in addition, one which had incurred suspicion. It contains one surprise: this is No. 34, a young stallion which was mated to two or three mares and only impregnated one of them successfully. But because one of the mares was unpromising on account of her own breeding record, there was little evidence against the horse. It can be concluded that all coefficients greater than 6.00 represent stallions whose efficiency as sires is poor.

On the assumption that, if full data were available, all stallions might be arbitrarily classed as *good*, *fair*, or *poor*, consideration of the remaining group becomes a fairly simple matter. The *poor* animals having literally segregated themselves, it must be composed mainly of those in the first two classes. It certainly contains the *good* ones. By reductive argument, those which would be called *fair* must be within its borders also. This class is a proportionately large one. It includes those average horses which are neither really good nor definitely poor; of necessity it is somewhat ill-defined. The point of practical value, however, is that as a whole, the animals in this class are reasonably satisfactory.

At this point it appears opportune to compare the results of the authors' observations with those already made on the sperm measurements of bulls (3). In the case of these animals, it was shown that, as a group, the thoroughly satisfactory ones had comparatively small coefficients of variation which did not vary greatly about a given mean. The coefficients of the *fair* bulls had a somewhat larger mean, and those of this group whose coefficients exceeded that mean became more and more suspicious as the function in question increased in value. Beyond a certain figure, all the coefficients represented *poor* animals.

In a general way the same statements appear to be true of horses. Aside from the figures themselves, the main point of difference is that it has not been possible to separate the good horses from the fair ones on the basis of their performance. From the evidence already in hand, the authors feel justified in assuming that, in this mixed group, the coefficients of largest size may warrant suspicion.

This is true in the case of stallion No. 3 from which two samples were obtained. One of them was *poor* (C = 6.51); the other was suspicious (C = 5.88). The animal's success as a sire had been anything but brilliant prior to the date of the first sample. He seems to have improved (?) somewhat by the time the second was forthcoming.

By analogy with the findings on bull sperm, and because of what is known concerning these horses, it is concluded, at least temporarily that, as measured, the coefficients of variation for good stallions have values between 4.00 and 6.00 (or less) their mean value probably being about 5.00.

One exceptionally good stallion from which three samples were obtained supports this conclusion rather consistently. As No. 8 in 1925, his coefficient was 5.46. The following year, as No. 29 and No. 30 his coefficients were 5.26 and 4.60, the samples being taken several weeks apart. Thus, from the probable mean of the coefficients for good horses, he exhibited a plus and minus variation of 0.46 and 0.40 respectively. But the degree of variation which an individual animal may normally show in the course of a year or a lifetime is without the possibilities of the present material.

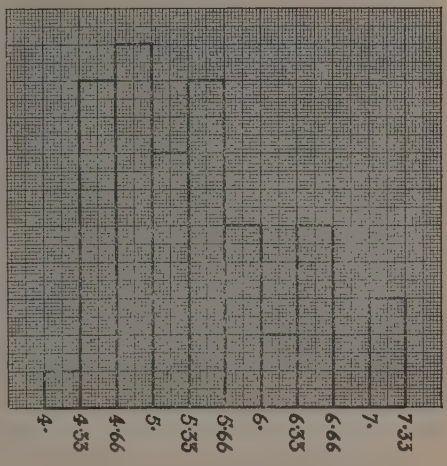

FIG. 1. *Histogram showing the distribution of the coefficients of variation for sperm head length in 51 samples of horse semen.*

Type of Curve

All the curves have been unimodal. With few exceptions they have also been symmetrical and essentially normal in type. There has been no opportunity to submit them to mathematical analysis, but it is fairly obvious from inspection that the curves of No. 30, 37, and 38 are somewhat asymmetrical or skew. The first and third of these animals would be classed as suspicious because of their high coefficients of variation (5.98 ± 0.127 and 6.00 ± 0.127); the second was definitely poor. For this reason, the authors are inclined to regard apparent skewness as a bad indication.

Head Length Dimorphism

The fact that no evidence of head length dimorphism was discovered in any of the slides renders the findings distinctly opposed to those of Wodsedalek (7) in particular, and to those of other biologists in general. An intensive study of Wodsedalek's data and the present results have convinced the authors that if there were approximately equal numbers of two types of sperm heads in semen, a dimorphic curve would probably result, providing the mean head lengths of these types differed by as much as 1 μ or more. Wodsedalek's curve shows a difference of 1.5μ and is therefore beyond impeachment on that score. But he worked with testicular sections, whereas the present work was done with ejaculated semen, and it is believed that the basis for the discrepancy between the two results lies in that fact. This point has already been elaborated elsewhere (3) and need not be repeated here.

Comparison of this Method and the Usual Cytological Examination

In the case of bulls, it has been shown that sperm head length variability studies conducted under high magnification often surpassed cytological examinations of the usual kind (at 800 to 1,000 diameters) as a means of detecting genital inefficiency. The reasons for this were two: (a), the unusual enlargement and accurate measuring made it possible to determine size variations which otherwise would not have impressed the eye; (b), the peculiar significance of the curves themselves, especially when of skew type. It should be noted, however, that the clinical values of most unusual cytological forms of cell had been rather well fixed prior to the variability studies being undertaken (5).

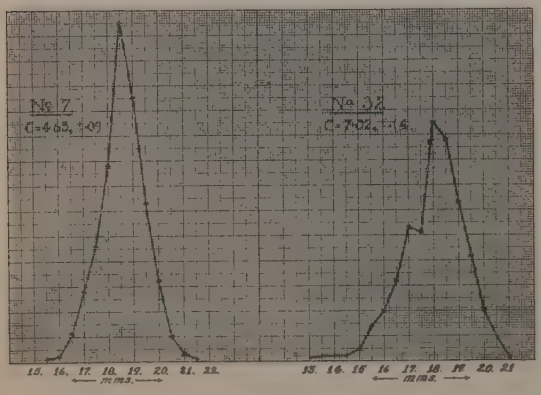

FIG. 2. *Frequency distribution curves of 500 sperm head lengths. No. 7 represents a good stallion: No. 32 a poor one. (magnification: 3000 ×).*

This was not the case in the present work. A systematic classification of morphologically aberrant horse sperms has never been undertaken. It seems that there is often a rather high proportion of somewhat unusual sperms even in the ejaculate of fairly satisfactory stallions. The reason for this cannot be stated at present, but the fact remains that until the cytology of horse semen is reduced to a more systematic basis, there is nothing very tangible with which this method can be compared. Time permitting, it is hoped to review the material in hand with the object of remedying this deficiency.

Conclusions

From the results obtained it is possible to conclude that:

1. Frequency distribution curves based on sperm head length are unimodal.

2. The coefficients of variation of these curves, in the case of normal animals range from 4.00 to 6.00 (or slightly less).

3. A coefficient of variation in excess of 6.00 indicates an unsound sire.

4. Skew curves have not been found in the case of normal animals, and may therefore be regarded with extreme suspicion.

5. In general, the results are in harmony with cytological data. But in order to be reduced to a systematic basis, both require further study, and it is not possible to compare them fully at present.

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PHYSICO-CHEMICAL STUDIES ON THE NATURE OF DROUGHT RESISTANCE IN CROP PLANTS[1]

By R. Newton[2] and W. M. Martin[3]

Abstract

Factors affecting drought resistance include those concerned in absorption, transpiration, and wilt endurance. The colloidal properties of leaf-tissue fluids are believed to be important in water retention under droughty conditions. These and other physico-chemical properties have been determined in a number of cereals, grasses and other plants, in relation to known drought adaptations. Methods for the study of colloidal properties have also been investigated.

Hydrophilic colloids bind water and increase the concentration of aqueous solutions, as shown by freezing-point determinations. Dextrose is less effective than sucrose in demonstrating this phenomenon. Concentration, quality, and state of dispersion or coagulation of colloids are factors affecting the degree of water binding.

The extraction of plant-tissue fluids and the estimation of dry substance by a modification of the refractometric method are described. The hydrolysis of sucrose added to plant juice in the bound-water determination is shown experimentally not to introduce serious errors. Storage of leaf tissue or press juice for a few hours even at 0° C. leads to changes in colloidal properties, but errors from this source are avoided by prompt handling.

Cylindrical dialyser sacs of 100 cc. capacity were found to require about 48 hr. for dialysis of plant juice at 3° C., with the water changed every hour. A gradual partial coagulation of colloids took place during dialysis, as shown by a decrease in refractive index, by an increase in gold number, and by sedimentation when centrifugalized. Hydration and dispersion were not always related, though both were affected by hydrogen ion concentration and by salts. Acid and alkaline salts stimulated hydration less because of the opposing "salt effect". In the presence of the natural crystalloids, hydration was usually greater than in dialysed juice.

The osmotic pressure of the tissue fluids of crop plants has been found to vary with physiological scarcity of water, but is not a reliable index of drought resistance. Bound-water content has been found more dependable; the cultivated wheats and several grasses have been on this basis satisfactorily arranged in the order of their drought resistance. The contrasting behavior of two poplar species, with reference to leaf fall and frost resistance, has also been explained. The separation of the colloids by dialysis and the determination of their gold number have been used as aids in interpreting the bound-water values.

The method of measuring imbibition pressure by direct pressure on masses of leaves proved unsuitable. The rates of water loss by evaporation from cactus segments and detached leaves of two grasses, under controlled humidity conditions, proved the remarkable ability of the cactus to retain moisture, but in the grasses showed no relation to drought resistance.

I. Introduction

The ability of certain plants to resist drought has long interested scientific observers, and the economic importance of this quality has furnished additional incentive to its investigation. In the semi-arid plains of Western America, where moisture is generally the most important factor limiting plant growth, plant breeders are giving a great deal of attention to the production of drought-hardy varieties of forage and cereal crops. For the identification of such

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varieties, reliance is commonly placed upon field observations of their behavior under droughty conditions. It seems possible that a greater knowledge of the fundamental nature of drought resistance might point the way to more rapid progress in breeding for this quality.

Investigations of xerophytism in the past have usually placed major emphasis on morphological and anatomical modifications, particularly in the leaves and stomata. While it is not intended here to discount the importance of such features in the adaptation of many plants to drought, it may be pointed out that they are not prominently developed in most of our common cultivated plants, and it seems possible that in these plants other aspects may be of greater significance.

The general method in the present investigation has been to determine the physico-chemical properties of the tissue fluids of a number of common crop plants, and consider the results in relation to the known adaptations of these plants to drought. The osmotic pressure of cell sap in relation to drought resistance has of course received a great deal of attention from many workers but, while this has been included as one of the standard determinations, in the present work more emphasis has been placed on colloidal properties. Osmotic pressure may be of more direct importance in absorption, but imbibition pressure seems likely to play a larger role in the retention of water under conditions of drought. Furthermore, an attempt has been made to measure the properties of the entire cell contents, that is, of sap and protoplasm together, rather than those of the sap only.

This investigation on drought resistance grew naturally out of earlier investigations on frost resistance (36, 37, 38) in which the similarities of these two properties had been observed and the importance to both of hydrophilic colloids had been pointed out. Under exposure to frost, desiccation occurs through water streaming from the interior of the cells to form ice crystals in the intercellular spaces; while under ordinary conditions of drought, the force of crystallization is replaced by the force of evaporation, and water is lost through vaporization into the intercellular spaces, whence it diffuses through the stomata into the atmosphere. It follows that any factor within the cell which opposes the abstraction of water will act as a resisting agent against physiological drought. The imbibition pressure of hydrophilic colloids is regarded as the most significant of such factors, and is assigned major importance in these investigations.

The plants studied included the following number of species or varieties: wheat, 9; oats, 8; barley, 4; rye, 3; cultivated hay grasses, 6; native wild grasses, 7; maize, 1; sunflower, 1; cactus, 1; poplar trees, 2. Those which are not cultivated crop plants were included because of special interest. The cultivated plants were grown in field plots under soil conditions made as nearly uniform as possible, while the native grasses were collected from habitats typical of each species.

The properties of the leaf-tissue fluids studied included: total solid content, crystalloidal content, hydrogen ion concentration, specific electrical con-

ductivity, osmotic pressure, colloidal content, gold number, ratio of colloids to crystalloids, and water-binding capacity of colloids of different species. In addition there were carried out a number of experiments with the tissues themselves, involving direct measurements of imbibition pressure and of the rate of water loss by evaporation under drying conditions; also a series of supplementary experimental studies relating to the theory and technique of the methods employed.

The investigations were carried on during the years 1924-25-26. In the following pages, all the experiments bearing upon a given point are assembled in one section, without strict regard for chronological order.

II. Review of Literature

That drought resistance is receiving much attention among scientific investigators is evidenced by the volume of literature appearing on the subject. Considerable of this has appeared during the progress of the work herein reported, and even since its completion. Among the recent contributions are some excellent and comprehensive reviews; reference may be made to those of Walter (52) and Maximov (30, 31). It will therefore be unnecessary here to do more than develop a general viewpoint of the problem, including those aspects which have been the special subjects of these investigations.

Plant Structure and Development in Relation to Absorption and Transpiration

Plants growing in desert regions have been of particular interest to those studying drought hardiness on account of their known drought-resisting properties. Xerophytic features mentioned by Schimper (47), such as reduced leaf surface, diminution of intercellular spaces, sunken stomata, thickened cuticle, surface hairs, and water storage cells are quite common to desert plants. These structural features function in mechanically reducing the rate of diffusion of water vapor from the mesophyll cells of the leaves into the external atmosphere. The studies of MacDougal (28), Cannon (8), Sabnis (46) Thoday (50), Bhide (5), and others (14, 29, 43) support the view that the ability of plants to withstand drought is usually closely associated with modified structural features. However, a review of the work just cited shows that structure is exceedingly variable, and that mechanical devices can be only partly responsible for the conservation of water in plants.

The classical work of Browne and Escombe (6) on the static diffusion of gases established the physical principles governing the escape of water vapor from plant cells to the outer atmosphere through the stomata. The transpiration experiments of Lloyd (27), Knight (23) and others have appeared to show, however, that stomatal movement cannot have more than restricted importance in the regulation of transpiration. Neither can conservation of water be effected satisfactorily by a reduction in size or number of stomata; such a modification would hamper gas exchange during periods of abundant moisture, thus limiting the rate of growth. This would not be in accord with observed

facts, for desert plants usually make rapid growth during moist periods, and merely maintain life processes during dry periods.

That the area of leaf surface is a factor in water loss was shown by experiments on the relative transpiration rates of corn and sorghum varieties, carried out by Miller and Coffman (34). This adaptation, like the one mentioned above, suffers from the objection that the rate of growth of varieties with reduced leaf areas would be limited during periods of abundant moisture by a low maximum rate of transpiration. To be effectively drought-hardy a plant should possess some means of regulating water loss according to moisture supply.

Bakke (3) concluded from his studies that transpiration rate may be taken as an index of xerophytism. On the other hand Iljin (21) compared the transpiration rates of two xerophytes, *Aster villosa* and *Veronica incana*, with two mesophytes, *Aristolochia clematitis* and *Sanguisorba officinalis*, growing under both dry and moist conditions. The xerophytes showed higher transpiration rates in both cases, indicating that under equal conditions, xerophytes cannot be distinguished from mesophytes by their relative transpiration rates. Iljin explains the difference in the water losses in the four species mentioned as being due to the fact that under drought conditions the stomata are closed in the mesophytes and open in the xerophytes.

A similar lack of relation between xerophytic structure and transpiration rate was observed by Maximov (32) and Wilson (55). The latter carried out a series of determinations on the relative transpiration rates of a large number of typical Australian xerophytic and mesophytic plants, and found that plants with most marked xerophytic features in some instances showed the highest rates of transpiration. It should, however, be pointed out that these transpiration rates were determined on plants growing in pots under good moisture conditions, and that had they been subjected to drought, different results might have been obtained. Nevertheless the results as they stand are in accord with those secured by Iljin.

Another instance in which structural features seem to have failed in reducing water loss is reported by Thoday (50), who observed a xerophytic plant, *Myrothamnus flabellifolia*, growing on granite slopes in Rhodesia. During drought periods the leaves of this plant become plicately folded and tightly adpressed to one another at the tips of the twigs, while the latter curve upwards. When moisture is restored the leaves and branches expand and growth is resumed. Notwithstanding this morphological feature, the moisture content of the plant during drought was observed to fall as low as 7%. Evidently the apparent xerophytic adaptation possessed by *Myrothamnus* is not effective in reducing water loss, and its resistance to drought must be due to some physiological adaptation which prevents a disorganization of the life processes during extreme desiccation. It is possible, however, that the structural arrangement functions in preventing rapid desiccation during the initial period of drought, thus enabling physico-chemical changes to take place in the cell fluids in such a way as to preserve the life processes during subsequent exposure.

The ability of certain desert plants to maintain life, without growth, at soil moisture contents even below the hygroscopic coefficient, was pointed out by Alway (2). He also found that among cultivated plants the interval between wilting and death varied from a few days in beans to many weeks in milo and wheat, the latter crops being more drought-resistant. This property has been strongly emphasized by Maximov (30, 31, 32), who considers the principal basis of drought resistance in plants to be their capacity for enduring a greater loss of water. In his experiments on wilting in relation to drought resistance, Maximov found that xerophytic plants were capable of losing half their water content without injury, whereas mesophytic plants showed a reduction in dry weight, depletion of food reserves and shedding of the leaves.

An analogy with frost resistance may be pointed out here. Newton and Brown (37, 38), in investigations extending over several winters, found that hardier varieties of winter wheat in general contained less moisture during the dormant season. This they regarded as a very important physiological adaptation. Plants which normally maintain a low moisture level during the frosty season would, by virtue of the greater concentration of the cell contents, offer a greater resistance to the abstraction of water from the cells by freezing. The cells would suffer also a smaller departure from the normal condition in attaining equilibrium with any given temperature below their freezing point.

Walter (53) distinguishes between transpiration and evaporation, as applied to loss of water from plants, defining transpiration rate as a property of the plant and evaporation rate as a property of the habitat. The greatest differences in evaporation rates he found not between dry and wet but between sunny and shady places. The conditions affecting evaporation changed quickly from place to place, so that it was not surprising to find xeromorphic and hygromorphic plants growing close together.

That plants develop more marked xerophytic features under droughty than under moist conditions is shown by numerous observations. Fuchsig (16) states that in acacias, the change from bipinnate leaf to phyllode is usually regarded as an adaptation to drought, a mode of protection against excessive transpiration. Most of the phyllode-bearing acacias are found in the dry regions of Australia and South Africa. When these plants are cultivated under moist conditions, reversionary shoots bearing pinnate leaves are produced. Cannon (7) irrigated a number of desert trees and shrubs, and found that in comparison with those grown in their dry native habitats, the irrigated plants in every case produced larger leaves and became more mesophytic in form. Thoday (50) observed more pronounced development of xerophytic features in *Passerina filiformis* when grown in exposed rather than in moist positions. Lee and Priestly (25) found experimentally that thickness of cuticle increases directly with a decrease in moisture and an increase in light intensity. Bews and Aitken (4), in studying the aëration systems in leaves of plants, observed that the power to vary the intercellular-space content means power to vary important physiological functions, such as transpiration, assimilation, and respiration. This view is in accord with the observed intercellular-space

content of mesophytic and xerophytic plants. As pointed out above, however, it is a fixed type of adaptation to drought, which does not provide for regulating transpiration according to the available moisture supply.

Another type of adaptation to drought pointed out by Moss (35) and others (9, 10) is the deciduous habit found alike in common trees and shrubs, and in certain desert plants. In winter when absorption is hindered by low temperatures, these plants reduce their transpiration rates to a minimum by shedding their leaves. This adaptation is more effectively xerophytic in some of the plants of desert regions, which produce a crop of leaves with each moist period and shed them again with the onset of drought. It often happens that several crops of leaves will be produced in a single year by these plants. Moss (35), in comparing the water relations of deciduous trees and the conifers, concluded that the deciduous habit was a more effective form of xerophily than the xerophytic leaf of conifers. The general xerophily of the latter, however, enables them to hold their own in the deciduous forests of the north temperate zone, and is essential in a region which for more than half the year is in a condition of physiological drought.

Root structure and development are important factors in absorption. Rotmistrov (45), Ivanov (22), Miller (33) and others (42, 43, 46) have shown that a highly developed root system increases efficiency of absorption and relative resistance to drought. Ivanov (22) observed that there was no direct relationship between transpiration rate and drought resistance, but that the ability of a plant to resist drought was directly proportional to the density and extent of root development. Miller (33) concluded that drought resistance in corn and the sorghums was proportional to the root development in relation to foliar area. Spalding (48) found that root-hair development in *Covillea tridentata* is usually proportional to water supply, and that schlerophyllous xerophytes usually possess high absorptive powers. As observed by Preston (42) and others, the highly developed root system of cacti in the surface layer of the soil enables them to absorb rapidly the moisture resulting from light showers, thus effectively using water which would otherwise be lost through evaporation from the surface of the soil.

Osmotic Pressure

Drabble and Lake (12) and Drabble and Drabble (11) first investigated the relation between the osmotic pressure of the cell sap in plants and their physical environment, and showed that osmotic pressure varied directly with physiological scarcity of water. Fitting (14) compared the osmotic pressures of plants of the same species collected in wet, dry and salty habitats in the Sahara Desert, and found they varied from about 7 to 100 atm. Maximum values for osmotic concentrations in higher plants are reported by Harris and his coworkers (19) who found plants of *Atriplex confertifolia* collected in salt flats near the Great Salt Lake in Utah with leaf-tissue fluids running from 74.2 to 153.1 atm. These leaves appeared to be in normal condition. In a plant of *Atriplex nuttallii* the value reached 169.3 atm. but there was some uncertainty as to whether the functional activities of the leaves were maintained under

these conditions. In this connection, on the assumption that the water content of the protoplasm stands in definite relation to the osmotic pressure of the cell sap, and that the latter in turn depends primarily upon the water balance in the plant, Walter (54) has recently sought to determine the adaptation of plants to drought by ascertaining the maximum cell-sap concentration which the plants can endure without permanent wilting.

Osmotic pressure probably functions mainly by increasing the absorptive power of plants, the greater concentration of the root sap enabling them to abstract moisture from relatively dry soils. The osmotic pressure of the leaf sap may also have an important effect on the upward flow of the transpiration stream. Drabble and Drabble (11) and Livingston (26) pointed out that an increase in the concentration of a solution does not reduce the vapor pressure enough to cut down effectively the rate of evaporation. On the other hand, a device which limits evaporation makes high osmotic pressure less necessary. Korstian (24) found greater sap densities in drought-resistant species in the Wasatch Mountains of Utah, except in those having thick cuticle or other epidermal coverings. Another device for resisting loss of water is discussed in the next subsection.

Imbibitional Properties of Cell Colloids

In his work on winter hardiness, Newton (36, 37) found the colloidal content of the cell fluids varied directly with relative hardiness, being high in the hardy and low in the non-hardy plants, and concluded that the colloids were important agents in resisting desiccation by freezing. He pointed out the fundamental relationship between the physical nature of cold resistance and drought resistance; also the two types of physiological adaptation to drought, depending upon high osmotic pressure and imbibition pressure respectively. As already noted, osmotic pressure is important chiefly in the absorption of water, while on the other hand, imbibition pressure may function in both the absorption and retention of water. In this connection Newton cites the observation of Harris and Gortner that a plant, *Atriplex nuttallii*, growing near Grantsville, Utah, with a freezing-point depression of 14.4° C., did not retain its water as effectively as did a cactus with a freezing-point depression of only 0.53° C. Juice of the latter was difficult to press out, and was extremely viscous, indicating a high colloidal content. Cell colloids apparently play an important part in the adaptation of the cactus to drought.

Spoehr (49) showed that the pentosan content of the cacti varies inversely with the water supply, a decrease in water supply being accompanied by an increase in pentosans, and *vice versa*. Pentosans are mucilaginous in character and have the power of swelling and imbibing enormous quantities of water. An increase in the pentosan content of the cells would therefore permit the absorption of large quantities of water during periods of abundance, and assist in retaining it against desiccation during drought periods. The water-storage capacity of the fleshy stems, together with the highly developed root systems described by Preston (42), enables the cacti to make very effective use of intermittent rains.

The water-retaining power of the cacti was determined experimentally by MacDougal (28), who found that in the absence of any water supply for two years, both *Echinocactus* and *Opuntia* produced growth; the latter even carrying out seed formation. The transpiration rate was determined on an uprooted *Echinocactus* in the absence of water supply over a period of 487 days with the following results.

Average daily water loss from 1st to 155th day = 6 gm.
Average daily water loss from 155th to 330th day = 3 gm.
Average daily water loss from 330th to 487th day = 2.3 gm.

These data indicate very approximately an imbibitional curve of a logarithmic type, similar to that obtained on drying emulsoid gels.

Another example in which colloids function in increasing absorptive powers and in resisting desiccation is reported by Price (43), who found on the roots of *Aristida pungens*, a desert grass of North Africa, a protective sheath formed by mucilage-secreting cells near the root cap. He pointed out that it is rare for a corky periderm to be formed by a monocotyledonous root, but that the mucilaginous sheath is an excellent substitute, not only protecting the roots against desiccation, but enabling the plant to absorb the least trace of moisture from the arid soil of the desert.

The same author showed later (44) that the resting spores of *Mucor* have their cell contents completely in the gel state. This physical condition is probably characteristic of very resistant forms. Fritsch and Haines (15) found that in most terrestrial algae, gelation of the protoplasts takes place only during drought, but in *Pleurococcus*, a very drought-resistant form, they are apparently always in this condition.

Gortner and Rude (18) showed that the percentage of bound water in plant-tissue fluids, the measure proposed by Newton and Gortner (40) for the content of hydrophilic colloids, was not closely correlated with the osmotic pressure or in fact with any of the other properties commonly determined. It appears to be a distinctive factor, the measurement of which may be expected to throw new light on the ecological relationships of plants.

Summary

The principal factors affecting drought resistance in plants are summarized diagrammatically in Fig. 1. Those concerned in absorption and transpiration are shown in some detail, but no attempt has been made to elaborate wilt endurance, the still obscure physiological adaptation which enables the plant to maintain life when the moisture content of the tissues becomes abnormally low.

The present investigations have been concerned mainly with the last group of factors shown under transpiration, particularly the colloidal properties of the leaf-tissue fluids, which it is believed are important in water retention and drought resistance.

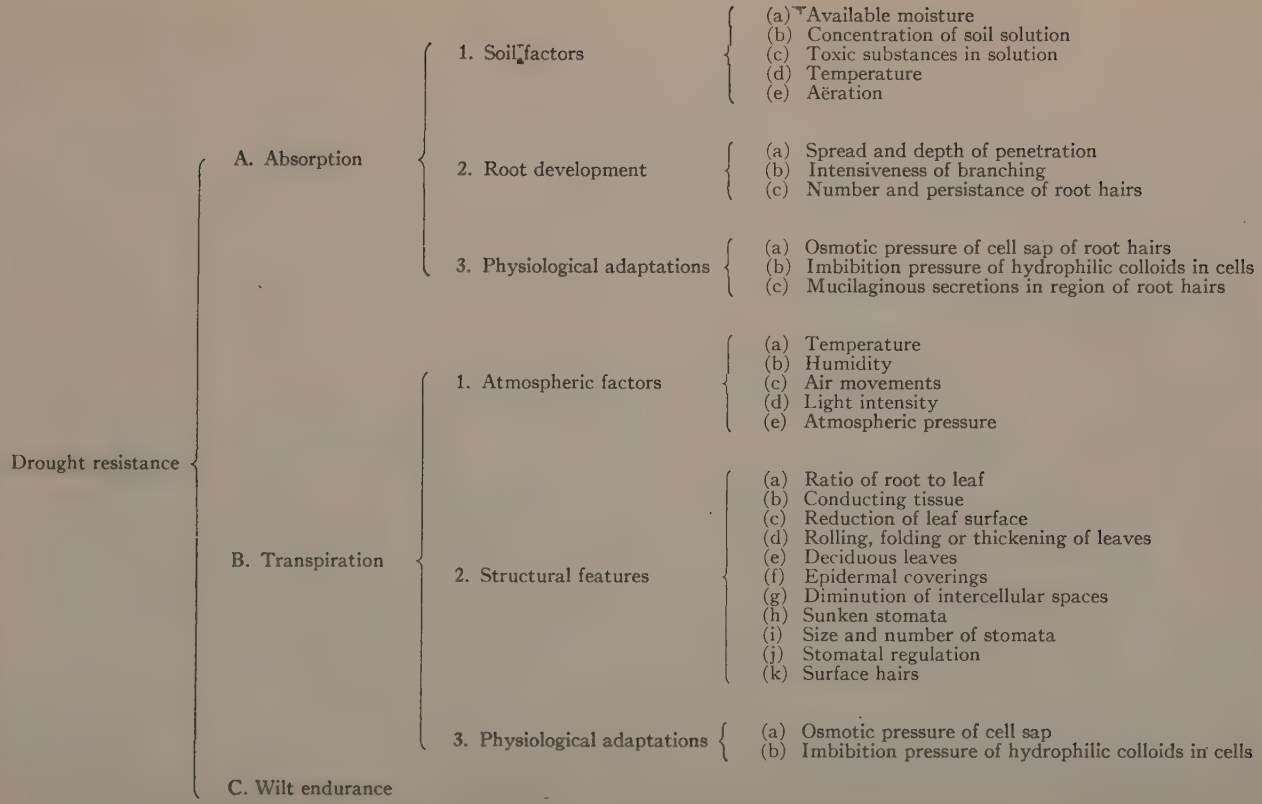
Drought resistance
A. Absorption
1. Soil factors
(a) Available moisture
(b) Concentration of soil solution
(c) Toxic substances in solution
(d) Temperature
(e) Aëration
2. Root development
(a) Spread and depth of penetration
(b) Intensiveness of branching
(c) Number and persistance of root hairs
3. Physiological adaptations
(a) Osmotic pressure of cell sap of root hairs
(b) Imbibition pressure of hydrophilic colloids in cells
(c) Mucilaginous secretions in region of root hairs
B. Transpiration
1. Atmospheric factors
(a) Temperature
(b) Humidity
(c) Air movements
(d) Light intensity
(e) Atmospheric pressure
2. Structural features
(a) Ratio of root to leaf
(b) Conducting tissue
(c) Reduction of leaf surface
(d) Rolling, folding or thickening of leaves
(e) Deciduous leaves
(f) Epidermal coverings
(g) Diminution of intercellular spaces
(h) Sunken stomata
(i) Size and number of stomata
(j) Stomatal regulation
(k) Surface hairs
3. Physiological adaptations
(a) Osmotic pressure of cell sap
(b) Imbibition pressure of hydrophilic colloids in cells
C. Wilt endurance

FIG. 1. *Diagram showing principal factors affecting drought resistance in plants.*

III. Bound Water as a Measure of Hydrophilic Colloids

When Newton and Gortner (40) proposed the bound-water method for estimating the hydrophilic colloid content of expressed plant-tissue fluids, the data they presented included a few readings obtained with artificial sols. These results showed that percentage bound water increased regularly with colloidal concentration, the graphical relation between the two suggesting an adsorption curve. Since that time considerable use has been made of the bound-water method in ecological studies (18), but no further experiments have been reported to prove the relation between bound water and concentration of colloids. Experiments on this point and on the technique of the determination were included in the present studies. The usual procedure followed in the determination of bound water in plant-tissue fluids will first be described.

The freezing-point depression of the fresh press juice is obtained by the Beckmann method, making the usual correction for undercooling. Then a fresh portion of juice containing exactly 20 gm. of water is weighed into a 100 cc. Erlenmeyer flask; this is a convenient quantity to permit the determination of the freezing point to be done in duplicate. In no case must the same portion of juice be frozen twice, since the frost precipitation of proteins alters the hydration and invalidates the results. The weight of juice required to contain 20 gm. of water is readily calculated from the previously determined content of dry substance as indicated by the refractometer; this, in fact, was conveniently accomplished by preparing a graph from which the weights were read directly. Since the principle of the method depends upon the determination of small differences in the amount of free water present in the juice, it is important that the juice used in the determination be weighed accurately. With the aid of a capillary pipette, prepared by drawing out one end of a piece of 8 mm. glass tubing and enlarging the opposite end so that pressure could be conveniently applied by the index finger to move the column of liquid in the capillary portion of the tube, juice samples could be weighed to within 0.001 gm. Precautions are necessary to keep dry the upper wall and neck of the flask. To the weighed portion of juice is added 6.8448 gm. of pulverized sucrose, just sufficient to make a molar concentration in the total water present. The sucrose samples are previously weighed into small aluminum dishes and stored in a desiccator until required. The aluminum dishes are provided with pointed lips to facilitate the transference of the sucrose to the flask containing the juice. The flask containing the sucrose and juice is stoppered and placed in a bath of ice slush on an electrically driven shaking apparatus. The latter was specially designed to give the mixture a gentle horizontal motion, to avoid frothing or physical changes in the colloids. The time required for the solution of the sucrose was found to be 20 min., when the latter had been ground so that it would pass through a 60-mesh sieve. The sifting was found to be important, as the time required for the complete solution of the sucrose is governed by the size of the largest particles.

The sucrose having been dissolved in the juice, its freezing-point depression

is again determined, and is usually found to be greater than the sum of the values for the juice and the sugar solutions determined separately. It is assumed from this result that not all of the calculated 20 gm. of water in the juice is in the free state, a certain quantity being held as imbibed water by the hydrophilic colloids; hence, that the amount of water available for the solution of the sucrose will be equal to 20 gm. less the quantity imbibed. The molecular concentration of the sugar will therefore be increased more than the theoretical amount, and this will be shown by an excess depression of the freezing point. The magnitude of the excess depression is taken as a measure of the water held in such a way as not to be available for the solution of the sucrose. The percentage of "bound water" is in turn taken as an index of the relative colloidal content of the press juice.

Fisher (13) has discussed critically the freezing of water in capillary systems. He pointed out that capillary water must be under considerable tension and hence may not have the same density as water in bulk. There seemed to be no doubt at least of the experimental fact of capillary water having a lower freezing point than water in bulk. Fisher also cited results to show that the freezing-point depression in a colloidal system was in all cases the sum of that due to capillarity and that due to the presence of solutes. Since this conclusion seems to exclude the effect of bound water in increasing the concentration of aqueous solutions and lowering the freezing point, it is important to note that all the work which Fisher cites was done with inorganic colloids, such as sand, soil and silica gels. That emulsoid colloids do in fact bind part of the water and so increase the concentration of added solutes may be proved by separating the phases (by filtering or centrifuging) and determining the freezing point of the solution in the absence of colloids or capillary water. This technique has been used extensively in this laboratory, in work on wheat flour, to be reported in another paper. Excess depression of the freezing point found by this method, since it cannot be due to the presence of colloids lowering the freezing point directly or to capillary water held under tension, must be due to differential adsorption of solvent and solute.

Comparison of Sucrose and Dextrose in Determination of Bound Water

In the original paper on the bound-water method there were stated the reasons for the choice of sucrose as a reference substance in the determination of bound water. Consideration had of course been given to other non-electrolytes of possible suitability, but either these were not easily available in satisfactory purity, or their properties in solution were unknown or objectionable. Urea, for example, was ruled out because of its effect on the hydration of proteins.

The main objection to the use of sucrose was the presence of invertase in plant saps and the apparent danger of obtaining false readings due to hydrolysis and consequent increase in molecular concentration. Under the experimental conditions then maintained, such an effect seemed to be negligible, a conclusion

which has been checked by more elaborate experiments to be reported in Section IV of this paper. At the outset of these new investigations, however, it seemed worth while to try dextrose, since if it proved satisfactory, it would entirely eliminate the above objection.

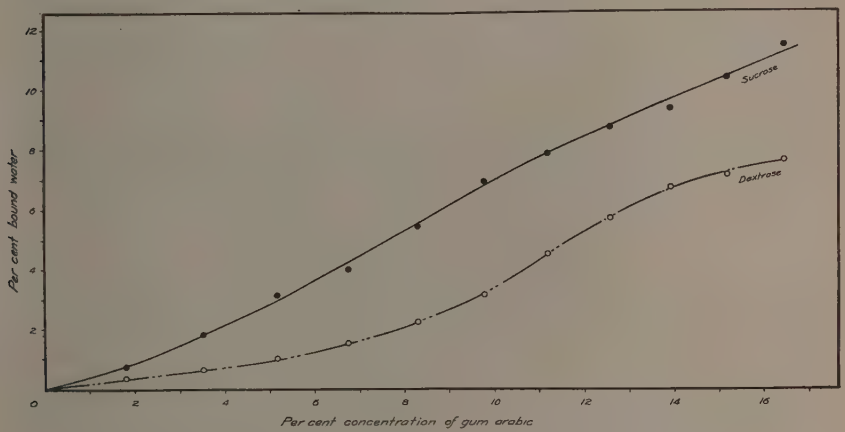

FIG. 2. *Comparison of sucrose and dextrose in determining bound water of gum arabic solutions.*

A series of solutions of various concentrations was prepared by dissolving finely pulverized gum arabic in distilled water at the temperature of melting ice. The solutions were allowed to stand at this temperature for 24 hr. after which the bound water was determined according to the method already described, using both sucrose and dextrose. The results are presented in Table I and Fig. 2. The discrepancy between the values given in the first two columns of the table, for concentration of the gum arabic solutions as made up by weight and as read later with the refractometer, is partly explained by the fact that the refractometer scale for solids is based upon concentration by volume. A more extended discussion of the estimation of dry substance by the refractometer, with additional data, is given in Section IV of this paper.

In Table I the freezing-point data are given in full to illustrate the method. To save space, they will be omitted from later tables, with the exception of the original freezing-point depression or corresponding osmotic pressure of the juice or solution, which is in itself an important property. The symbols used as headings in the table have the following significance:

$\triangle$ = Freezing-point depression of the gum arabic solutions.

$\triangle_a$ = Freezing-point depression of the solutions after a molar concentration of sugar had been added.

$\triangle_s = \triangle_a - \triangle$, the additional depression due to the added sugar.

$\triangle_x = \triangle_s$ *minus* the freezing-point depression (determined experimentally) of a molar solution of the sugar used.

The percentage bound water, determined with sucrose, is equal to $\frac{\triangle_x \cdot 89.2}{\triangle_s}$,

where 89.2 is the percentage of free water in a molar solution, assuming the formation of sucrose hexahydrate, as explained in the original paper (40). The freezing-point depression of the "c.p." sucrose in distilled water was always slightly in excess of that expected on the foregoing assumption, owing either to slight impurity or to the formation of a higher hydrate. Every new stock was carefully tested by a large number of determinations, and sufficient was mixed and ground at one time to avoid having to change the stock more than once a year.

The "c.p." dextrose, on the other hand, gave less than the theoretical freezing-point depression in molar concentration in distilled water, even assuming no hydration of the sugar; consequently the percentage bound water in this case is taken as equal to $\frac{\triangle_x \cdot 100}{\triangle_s}$.

TABLE I

COMPARISON OF SUCROSE AND DEXTROSE IN DETERMINING BOUND WATER IN GUM ARABIC SOLUTIONS OF VARIOUS CONCENTRATIONS

Concentration gum arabic solution (by weight) %	Concentration by refractometer (by volume) %	$\triangle$ °C.	Determined with sucrose				Determined with dextrose			
			$\triangle_a$ °C.	$\triangle_s$ °C.	$\triangle_x$ °C.	Bound water %	$\triangle_a$ °C.	$\triangle_s$ °C.	$\triangle_x$ °C.	Bound water %
1.79	1.9	0.015	2.166	2.151	0.017	0.7	1.827	1.812	0.008	0.4
3.50	3.6	0.035	2.212	2.177	0.043	1.8	1.849	1.814	0.010	0.6
5.16	5.4	0.046	2.257	2.211	0.077	3.1	1.868	1.822	0.018	1.0
6.75	7.2	0.053	2.288	2.235	0.101	4.0	1.884	1.831	0.027	1.5
8.28	8.8	0.062	2.335	2.273	0.139	5.4	1.907	1.845	0.041	2.2
9.76	10.4	0.073	2.385	2.312	0.178	6.9	1.935	1.862	0.058	3.1
11.19	12.0	0.084	2.422	2.338	0.204	7.8	1.973	1.889	0.085	4.5
12.56	13.5	0.096	2.462	2.366	0.232	8.7	2.009	1.913	0.109	5.7
13.90	15.0	0.114	2.498	2.384	0.250	9.3	2.048	1.934	0.130	6.7
15.18	16.4	0.132	2.546	2.414	0.280	10.3	2.073	1.941	0.137	7.1
16.43	17.8	0.148	2.594	2.446	0.312	11.4	2.100	1.952	0.148	7.6

The results show that the bound-water values obtained with sucrose were much greater than those obtained with dextrose. Therefore sucrose was used throughout these investigations, with the exception of some work done during the early part of the first season (1924) before the relative suitability of the two sugars was known. The results then obtained with dextrose may be compared in Table XVII with results obtained on similar tissue fluids with sucrose, and serve to show again the superiority of sucrose in demonstrating bound water. In one case not reported in the table the bound water of Kubanka wheat leaves, collected July 10, 1924, was determined with both dextrose and sucrose, on separate portions of the same sample of juice, and gave values of: dextrose, *nil;* sucrose, 5.3%.

Apparently some of the imbibed water which was not available for the solution of sucrose, was available for the solution of the dextrose. It is possible, however, that dextrose may be more readily adsorbed by colloidal surfaces than sucrose, a supposition which is strengthened by the fact that the latter

is hydrated in solution whereas the former apparently is not. Whatever the explanation may be, it is evident that sucrose will give a more sensitive test, and it is therefore more suitable for the determination.

Effect of Sucrose Concentration on Bound-water Values

When sugar is added to a solution of a hydrophilic colloid, there must presumably be established an equilibrium between the imbibitional forces of the dispersed particles, and the osmotic force tending to abstract water. It seemed possible that the molar concentration of sugar used might cause a measurable reduction in the hydration of the colloidal particles, and that a lower concentration might demonstrate a larger amount of bound water.

An experiment was carried out with a gum arabic solution of 13.47% concentration, to portions of which was added sucrose in 0.5, 1.0, and 1.5 *M.* concentrations. In calculating bound water it was assumed that the sucrose formed the hexahydrate in all three concentrations, and that the percentages of free water in the sugar solutions alone would therefore be 94.6 in 0.5 *M.*, 89.2 in molar, and 83.8 in 1.5 *M.* The bound-water values found were: 0.5 *M.*, 8.5%; 1.0 *M.*, 9.5%; 1.5*M.*, 6.3%. These are in each case the average of six determinations with a maximum spread equal to 0.3% bound water.

The results obtained with molar and 1.5 *M.* concentrations of sugar bear out the suggestion put forward above. That found with half-molar concentration, however, is out of line, since it seems to show less rather than more hydration of the colloids as compared with that found in molar concentration. It is difficult to accept this conclusion, and one is inclined to suggest that some other factor must have affected the results. Nevertheless, the experiment made it appear advisable to adhere to the molar concentration in the regular procedure, and this accordingly was done.

Relative Water-binding Capacity of Different Colloids at Various Concentrations

To obtain further information on the relation of bound water and concentration of colloids and also to establish some basis of comparison between the water-binding capacity of colloidal sols of known composition and the complex hydrosol in the plant cell, an experiment was carried out with a number of colloidal substances easily procurable in commercial form.

The colloids used included gelatin, agar, vegetable albumin, dextrin, and blood fibrin. A series of solutions of various concentrations was prepared by adding the materials, ground to pass a 100-mesh sieve, to distilled water and storing for 24 hr. in an ice-bath on the shaker previously described, after which the bound water in each was determined. The upper limit of concentration in each case was set by the viscosity beyond which it was no longer practicable to carry out the determinations. The results are given in Table II and Fig. 3, including for comparison the values found for gum arabic solutions with sucrose, brought forward from Table I.

These colloids show considerable variation in the water which they bound under the conditions of this experiment. It should be pointed out that only

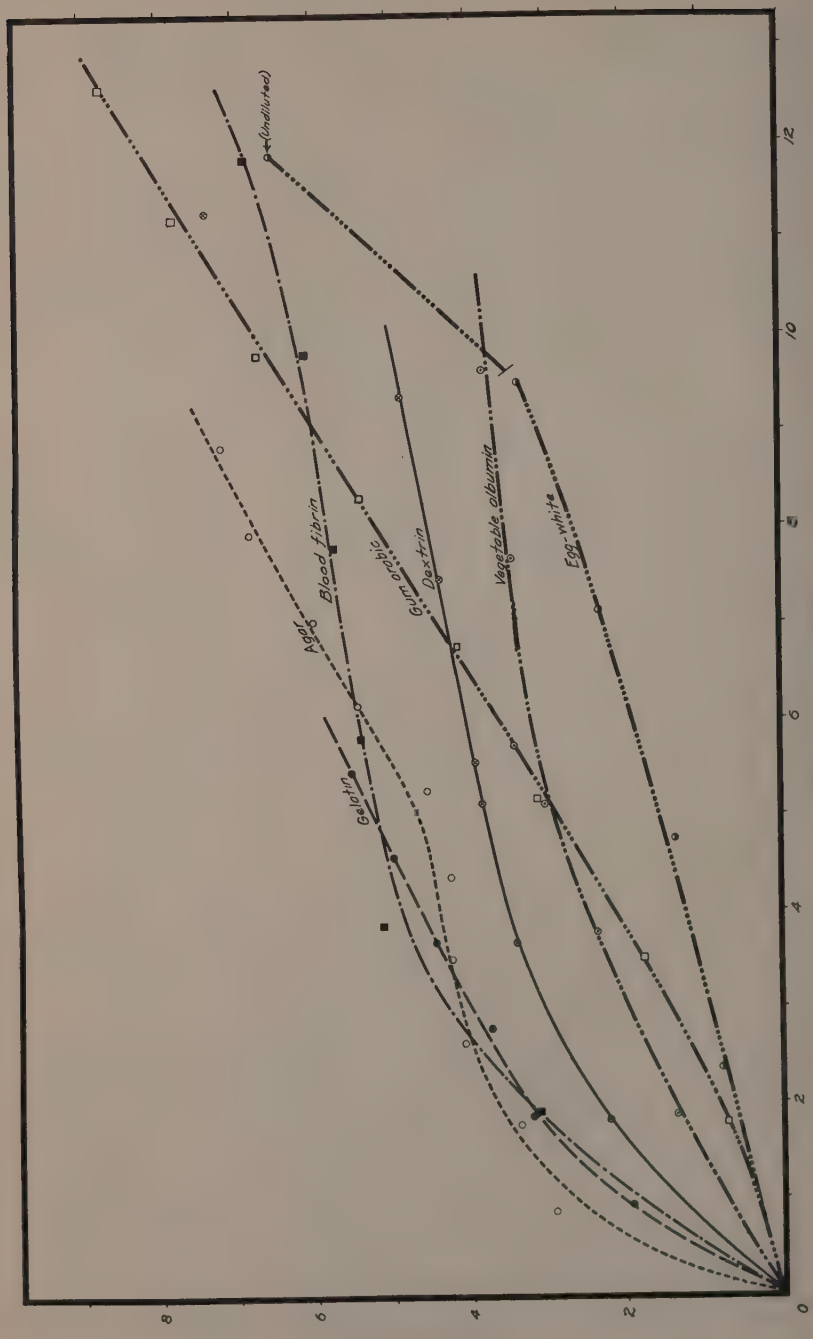

FIG. 3. *Water bound by different colloidal substances at various concentrations.*

TABLE II

WATER BOUND BY DIFFERENT COLLOIDAL SUBSTANCES AT VARIOUS CONCENTRATIONS

Material	Actual concentration %	Concentration by refract. %	△ °C.	Bound water %	Bound water per gm. colloid gm.
Gelatin	0.93	0.3	0.002	1.9	2.05
	1.86	0.5	0.004	3.2	1.70
	2.77	0.9	0.006	3.7	1.31
	3.66	1.4	0.005	4.4	1.17
	4.55	1.6	0.006	5.0	1.04
	5.43	2.4	0.010	5.5	0.96
Agar	0.87	0.1	0.003	2.9	3.36
	1.77	0.3	0.013	3.4	1.87
	2.61	—	0.020	4.1	1.52
	3.48	—	0.029	4.2	1.17
	4.36	—	0.036	4.2	0.93
	5.24	—	0.047	4.5	0.82
	6.13	1.0	0.054	5.5	0.84
	7.02	1.1	0.060	6.0	0.79
	7.92	—	0.064	6.8	0.79
	8.81	—	0.067	7.2	0.74
Vegetable albumin	1.88	—	0.027	1.3	0.70
	3.78	—	0.041	2.3	0.60
	5.11	0.2	0.046	3.0	0.56
	5.70	—	0.055	3.4	0.56
	7.65	—	0.068	3.4	0.41
	9.62	—	0.080	3.8	0.35
Dextrin	1.83	—	0.050	2.2	1.19
	3.67	—	0.062	3.4	0.89
	5.12	5.4	0.102	3.8	0.71
	5.54	—	0.095	3.9	0.66
	7.43	—	0.120	4.3	0.54
	9.34	—	0.148	4.9	0.47
	11.27	—	0.182	7.0	0.55
Blood fibrin	1.91	0.0	0.014	3.1	1.61
	3.84	0.0	0.020	5.1	1.29
	5.79	0.0	0.032	5.4	0.88
	7.77	0.0	0.042	5.7	0.68
	9.78	0.0	0.052	6.1	0.56
	11.81	0.0	0.062	6.9	0.51
Gum arabic	1.79	1.9	0.015	0.7	0.38
	3.50	3.6	0.035	1.8	0.48
	5.16	5.4	0.046	3.1	0.57
	6.75	7.2	0.053	4.0	0.56
	8.28	8.8	0.062	5.4	0.60
	9.76	10.4	0.073	6.9	0.64
	11.19	12.0	0.084	7.8	0.62
	12.56	13.5	0.096	8.7	0.61
	13.90	15.0	0.114	9.3	0.58
	15.18	16.4	0.132	10.3	0.58
	16.43	17.8	0.148	11.4	0.58

dextrin and gum arabic were completely dispersed by the treatment with ice water. This was shown both by the appearance of the mixtures and by the refractometric readings. Only about a third of the gelatin and a sixth of the

agar went into solution, as indicated by the refractometer, while of the blood fibrin no detectable amount dissolved. The appreciable solubility of ground gelatin in cold water is in line with the behavior of this substance found by Alsberg and Griffing (1). Nevertheless, the relative insolubility of the substances did not prevent them binding water, since it will be seen in Fig. 3 that the graphs for gelatin, agar and blood fibrin are the highest in the series. There is perhaps no reason to suppose that true imbibition, or the adsorption of water on the interior surfaces of colloidal particles, cannot go on to the same extent with large particles as with small ones if given time, provided the non-dispersion of the particles is not due to irreversible coagulation which would prevent swelling.

In most cases the bound water per gram of colloid tends to diminish with the concentration of the sols. Gum arabic is an important exception, in which hydration seems not to be affected in this way, and over most of the range used bound water is almost a linear function of concentration. That this behavior is characteristic of all samples of gum arabic must not, however, be concluded, since the original experiments on which the bound-water method was based (40) indicated a logarithmic relation between bound water and concentration of this substance. The special case of egg white (included in Fig. 3, but not in Table II) will be discussed later. Whatever may be the exact mathematical expression of the relationship in different cases, the results as a whole seem to prove conclusively that bound water is in fact closely associated with concentration of hydrophilic colloids.

A comparison of the water bound per gram of colloid in these prepared sols with that found in the tissue fluids of various plants, shows higher values in the natural sols in the plant cell. The latter data will be given in detail in another section, but as an example it may be stated here that the press juice of cactus plants containing 5.1% dry substance showed 16.6% bound water, or 3.09 gm. per gram dry substance, equivalent to an average hydration of 309%. This is equalled by only one value in Table II, that found in the most dilute agar sol, and by comparison with other figures in the same series it seems likely that this agar value is too high, owing probably to experimental error. It must be remembered also that in the case of the cactus the total dry substance was included, crystalloids as well as colloids. Still higher values were found in the press juice of certain grasses, when the colloids only were considered. On the other hand, it must be pointed out that in the preparation of the artificial sols no effort was made to adjust hydrogen ion concentration or other factors which might affect hydration. Data will be presented later to show that in the presence of the mixture of electrolytes in the natural sols extracted from plant tissues, hydration of the colloids is usually greater than when these are separated by dialysis. Nevertheless the conclusion is justified that the colloidal substances in plant cells compare favorably in imbibitional capacity with various other substances well known for their hydrophilic properties.

The specific electrical conductivity was later determined on the materials used in the above experiment, to get some suggestion as to the content of

electrolytes. The determination of conductivity, wherever reported throughout this paper, was carried out with a highly accurate Wheatstone bridge outfit, with the solutions at $25 \pm 0.05°$ C. The Zsigmondy gold number was also determined on three of the substances. This is a method of estimating the quality of colloids which has been applied to natural plant sols in our studies of winter hardiness (37), and has been used again in the present investigations.

TABLE III

SPECIFIC CONDUCTIVITY AND GOLD NUMBER OF VARIOUS COLLOIDAL SUBSTANCES

Substance	Concentration %	Conductivity at 25° C. $K \times 10^3$	Gold number	
			Dissolved at 0° C.	Heated to 65° C. for 30 min.
Gelatin	5.12	0.89	0.011	0.007
Agar	5.12	0.88	—	—
Vegetable albumin	5.12	0.52	—	—
Dextrin	5.12	0.54	17.946	20.937
Blood fibrin	5.12	0.45	—	—
Gum arabic	5.12	1.29	0.075	0.075

These additional data, given in Table III, show that the electrolyte content was in all cases very low. That of gum arabic, however, is significantly higher than the others, and it is not impossible that this fact may be connected with its different behavior in regard to the relation between bound water and concentration. A comparison of its freezing-point depression with that of the sample used earlier (40), which had behaved more like these other colloids, shows that the new sample contained about twice the quantity of crystalloids present in the old.

Since the data in Table II show that total bound water increases with concentration of any colloidal substance, and that the bound water per gram at any concentration varies considerably with different substances, it appears that the water-binding capacity of any sol is a function of both quantity and quality of colloids present. For the measurement of quantity in plant juices we had recourse to dialysis, and for quality, as already intimated, to the gold number. The latter represents the number of milligrams of the substance required to protect the particles in 10 cc. of a red gold sol from aggregation under the influence of 0.1 gm. of salt. The protective quality of the colloid is therefore inversely proportional to the size of the gold number. That the protective quality is related also to water-binding capacity is suggested by various data throughout this paper, including the values for gelatin, dextrin and gum arabic given in Table III. This is true at least of gelatin and dextrin. The data for gum arabic are difficult to interpret, on account of the abnormal shape of its water-binding curve (Fig. 3), which has been discussed above. The marked difference in the values for gelatin dissolved in ice water and that peptized by heat is of course due to incomplete solution in the former case.

Bound Water and Other Properties of Egg White and Egg-white Solutions

Egg white, being a conveniently obtainable natural colloid, was used in an extension of the foregoing experiments with various substances, its bound water, specific conductivity and hydrogen ion concentration being determined, together with the effect of dilution on these properties.

The whites of 16 fresh eggs were removed and thoroughly mixed, and the content of dry substance was determined by the refractometer. Then to purify the mixture, it was beaten, allowed to settle for 3.5 hr, and the froth removed. The dry substance of the fluid was again determined. A portion of the froth still remaining was removed and allowed to settle for another hour, after which the dry substance of the fluid so obtained was read on the refractometer. In all cases the refractometer readings were repeated several times on different samples to ensure accuracy. The effects of frothing on the concentration of the egg white are shown in the following values:

Concentration before frothing.....15.3%
Concentration after frothing and settling for 3.5 hr.....15.0%
Concentration of fluid settling from froth removed after 3.5 hr and settling for one hour more.....15.5%

Evidently there was an accumulation of dissolved substances in the froth or surface films in accordance with the Gibbs-Thomson principle.

The purified egg white was next diluted to various concentrations and the bound water, specific conductivity, and hydrogen ion values determined. The methods for the first two determinations have already been described; for hydrogen ion concentration, here and throughout these investigations, a Leeds and Northrup type K potentiometer and a bubbling hydrogen electrode were used. The results with egg white are presented in Table IV and Fig. 4, those for freezing-point depression being shown in the figure as the corresponding osmotic pressure. The last line of values in the table and those at the right side of the figure are for undiluted egg white. The bound-water curve for egg white is included also in Fig. 3.

TABLE IV

Bound water and other properties of egg white and egg-white solutions

Conc'n by drying* in %	Conc'n by refractometer in %	Δ in °C.	Osmotic pressure in atm.	Conductivity at 25° C. $K \times 10^3$	H ion conc'n pH	Bound water in %
2.37	3.0	0.096	1.16	2.25	8.48	0.7
4.74	6.3	0.198	2.39	—	—	1.3
7.11	9.2	0.294	3.54	5.72	8.48	2.3
9.48	12.3	0.389	4.69	—	—	3.3
11.85†	15.0	0.471	5.67	8.26	8.45	6.5

Only undiluted egg white †dried; other values in this column calculated from dilution.

Osmotic pressure and specific conductivity behaved normally upon dilution,

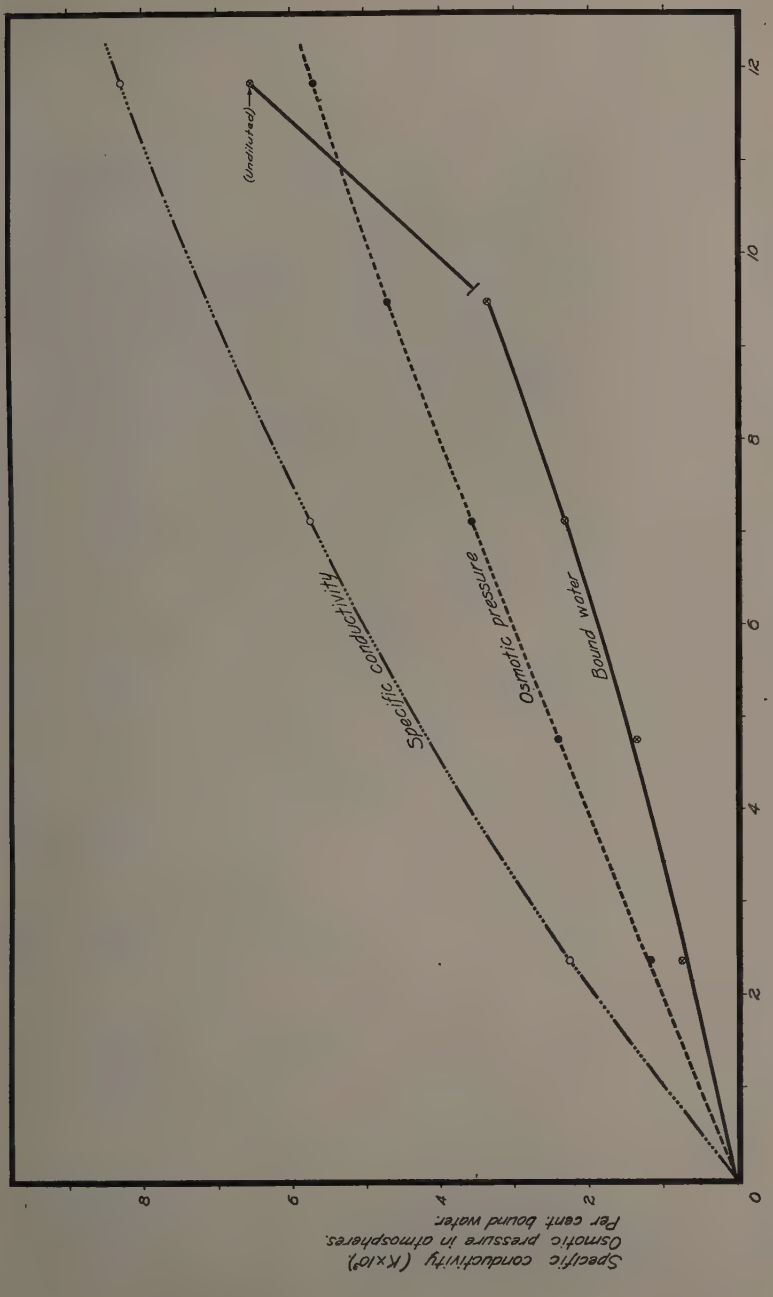

FIG. 4. *Bound water, osmotic pressure, and specific conductivity of egg white and egg-white solutions.*

the latter showing a more pronounced curve because more affected by the increased dissociation of electrolytes; that is, the effect of increased dissociation on the osmotic pressure is partly masked by that of the non-electrolytic crystalloids.

On the other hand, the initial dilution of the egg white caused a sudden drop in the bound water. With the addition of water the egg white became markedly opalescent, the globulin apparently being thrown out of solution with a consequent decrease in the average hydration of the colloids present. Further dilution caused less pronounced changes, yet the whole bound-water curve is in shape quite the reverse of an adsorption curve which most of the prepared colloids tended to approach (Fig. 3). Since there was no change in hydrogen ion concentration, the effect must be due simply to changes in solubility with diminishing salt concentration.

It will be shown later that the progressive removal of electrolytes from plant juices by dialysis causes a gradual coagulation of the colloids present. It is mentioned here because of its analogy to the behavior of egg white on dilution, and in order to point out an important relation to methods for the extraction of the fluid constituents of fresh plant tissues. If water is used in the process, as is quite common, it must not be expected that the proteins will be obtained in an unchanged physical condition.

Summary

When sucrose is added to a solution of a hydrophilic colloid, such as plant juice, the freezing-point depression is usually increased more than the theoretical amount, owing to part of the water being "bound" by the colloid. Dextrose is much less effective in demonstrating this phenomenon. Sucrose gave best results for this purpose when used in molar concentration.

The total water bound by finely ground gelatin, agar, blood fibrin, vegetable albumin, dextrin and gum arabic, when added to cold water, increased with the concentration of the colloid. The water bound per gram, in all but the gum arabic, decreased with concentration. These substances showed considerable variation in water-binding capacity, but none were equal in this respect to the colloids of certain plant juices. Both quality and quantity of colloids come in question in this respect. The gold number is suggested as one measure of quality.

The bound water in fresh egg white decreased disproportionately on dilution with water, owing to the globulin being thrown out of solution. A similar coagulation of plant-juice proteins on removal of electrolytes by dialysis has been observed.

IV. Application of Bound-water Method to Plant-tissue Fluids

In beginning these investigations considerable time was necessarily spent in developing technique and in overcoming some of the difficulties encountered. The rigid standardization of all methods was found to be highly important, in order that results obtained with different tissues might be comparable.

In this section are described experiments checking various points in the application of the bound-water method to plant-tissue fluids.

Collection of Samples and Preparation of Press Juice

The cultivated plants used in these investigations were grown in field plots under soil conditions made as nearly uniform as possible, while the native wild plants were for the most part collected from habitats typical of each species. On account of the well-known variation in the osmotic pressure of plant saps throughout the day, collections were made at approximately the same hour each morning. It is impossible, of course, to control the fluctuations occurring from day to day due to changes in weather and soil conditions, but species which it was intended to compare were collected in as compact a series as possible with respect to date, and comparisons based generally on several series of collections made throughout the season. Only green leaf tissue was used. When spring cereals are shown in various tables in this section as being collected in late summer or fall, the collections were made from plots sown late for the special purpose of these experiments.

The leaves as collected in the field are placed directly in quart Mason jars packed in buckets of crushed ice, and removed to the laboratory in this condition. In the grinding and pressing which follows, all apparatus which comes in contact with the tissues and juice is chilled before use to about 0° C. A portion of about 350 to 400 gm. of sound tissue is ground in an ordinary meat grinder, adjusted to cut as finely as possible. The pulp is wrapped in a single thickness of heavy cotton (previously boiled and rinsed in distilled water, and dried before use), placed in a steel press bowl 12.5 cm. in diameter with perforated walls and a gutter base, and hydraulic pressure applied very gradually, just enough to keep the juice flowing slowly but not enough to move the pointer of the pressure gauge away from the zero stop. The pointer actually does not move until a pressure of about half a ton has been reached, equal in terms of unit area of the piston to about three or four atmospheres. When the pointer does begin to move, no further pressure is applied, nor is any attempt made to secure the fluid remaining in the pulp. From the gutter base of the press bowl the juice flows through a short length of rubber tubing into a flask packed in ice. The last operation is to whirl the juice four minutes in centrifuge tubes jacketed with ice slush, with the rheostat governing the speed of the centrifuge (International No. 2) set at the 8th stop.

The experiments on which the foregoing standard method of extracting the tissue fluids was based have already been described in another paper (39). The important points to note are: (a) that it is speedy; (b) that tissue and juice are kept chilled at all stages; (c) that no pretreatment such as freezing is given the tissue; (d) that heavy pressures are avoided; (e) that no solvent is added, not even water. Deviation from any of these practices is shown, either in this paper or in that just cited, to lead to changes in the juice and, at least from the point of view of these investigations, to unrepresentative results.

Estimation of Dry Substance in Plant Juices by Refractometer

The bound-water method calls for weighing out a sample of fluid containing a known weight of water, to which can then be added an exact molar concentration of sugar. In order to be able to do this with plant juice immediately after it is expressed, it is necessary to have some way of reading the content of dry substance directly. Gortner and Hoffman (17) proposed, for this purpose, the use of a refractometer equipped with a sugar scale, on the assumption that the refractive index of the average mixture of substances in plant saps would be near enough to that of sucrose to avoid excessive errors. In an earlier paper from this laboratory (38) it was pointed out that to make the results comparable with those obtained by oven-drying the juice of winter wheat plants, it was necessary to reduce the refractometric readings by about 15%. In the present investigations it was discovered that with most summer tissues this correction was insufficient. In a few cases bound-water determinations gave negative values, showing that more than the calculated amount of solvent was present. It was therefore decided to investigate further the relation between dry substance as indicated by the refractometer and by other methods.

By oven-drying, volatile materials other than water are undoubtedly lost. These, however, probably constitute but a small proportion of the dissolved substances. Further, as they may act as solvents for sucrose, from the point of view of the bound-water determination they are perhaps more legitimately included with the water than with the dry substance of the juice.

The determinations were carried out by drying 10 gm. portions of juice in aluminum dishes of 5 cm. diameter, in triplicate, first in a ventilated oven at 60° to 63° C. for 72 hr., then in a vacuum oven at 96° to 97° C. for 48 hr. This excessive length of drying was done because of the well-known difficulty of driving off the last traces of moisture from the gummy residues left by plant saps.

The results presented in Table V show that on the average the plant juices contained 83.0% of the dry substance indicated on the sugar scale of the refractometer. It should be explained, however, that the refractometer scale is graduated to read the number of grams dry substance in 100 cc. liquid, whereas the oven-drying data are given on the basis of grams dry substance in 100 gm. liquid. The divergence between the two sets of figures is therefore slightly greater than it would be if they were both expressed on the same basis. Nevertheless, in the bound-water method the interest lies in the actual weight of water in a given weight of juice, so that any correction of the refractometer readings must be based on the values as given in the table. A correction of 15.3%, based upon the earlier work, was actually used in all the bound-water determinations carried out with plant juices in 1924, and such values reported for that season are in consequence probably slightly low. In 1925 and 1926, the correction used was 17%.

The prepared sols used in various experiments were normally made up by weighing out both the dry substance and water before mixing. Methods for determining the dry substance of the sols therefore did not come in question.

TABLE V

DRY SUBSTANCE IN PRESS JUICE AS DETERMINED BY REFRACTOMETER AND OVEN-DRYING

Material	Date collected 1924	By refractometer %	By oven-drying %	Per cent of refractometric reading found by oven-drying
Triticum vulgare, var. Marquis	July 3	10.4	8.82	84.8
T. monococcum, Einkorn	July 3	13.2	11.18	84.7
Hordeum vulgare var. Barks	July 3	9.2	7.79	84.6
H. distichon var. Canadian thorpe	July 3	8.7	7.66	88.1
Avena sativa var. Banner	July 3	9.7	7.55	77.8
A. sterilis var. Red	July 3	9.6	7.94	82.7
Triticum vulgare var. Marquis	Sept. 10	9.6	7.96	82.9
T. durum var. Kubanka	Sept. 10	9.6	7.90	82.3
Avena sativa var. Banner	Sept. 10	8.5	7.41	87.2
A. strigosa, Sand oats	Sept. 10	10.4	8.75	84.1
Helianthus annuus var. Russian giant	Sept. 10	11.2	9.29	82.9
Zea mays var. Northwestern dent	Sept. 10	13.3	9.06	80.2
Secale cereale var. Alberta winter	Sept. 10	14.9	11.96	80.3
Agropyron tenerum, Western rye grass	Sept. 11	21.0	16.79	79.9
Phleum pratense, Timothy	Sept. 11	9.6	8.03	83.7
Hordeum vulgare, var. O.A.C. No. 21	Sept. 11	8.9	7.34	82.5
H. vulgare plus 5% sucrose	Sept. 11	13.4	11.09	82.8
				Av. 83.0

However, because of its interest in relation to the method used for plant juices, refractometric readings were usually made for comparison, and these appear in Tables I, II and IV. In addition to this, one special experiment was carried out in which various sols were dried either in a vacuum oven or in a vacuum desiccator over concentrated sulphuric acid. Their concentrations as determined by drying are compared in Table VI with that indicated by the refractometer. The values obtained by drying are in this case expressed as the number of grams dry substance in 100 cc. liquid, to make them comparable with the refractometric readings.

The gelatin used in this experiment was peptized by heat, but the other substances dissolved readily in cold water. Fresh egg white was used. The results indicate as was expected that the refractive index varies according to the composition of the material in solution. The two methods give more concordant values with the carbohydrates than with the proteins. This is not surprising, since the scale on the refractometer is graduated for sucrose. The results with gelatin suggest that the refractive index is not always a linear function of concentration. That this divergence is not, however, necessarily characteristic of proteins is shown in the more extended series with egg white reported in Table IV.

The results as a whole show that, if the refractometer is employed in determining the dry substance in heterogeneous mixtures such as plant juices, it is essential to find experimentally the proper correction. It has been assumed in these investigations that the same correction might be employed for the juices of nearly related plants like the various cereals, collected during the active

TABLE VI

CONCENTRATION OF COLLOIDAL SOLS AS DETERMINED BY REFRACTOMETER AND DRYING

Material	Method of drying	Concentration by drying in %	Concentration by refractometer in %	Dry weight as % of refractometric reading
Gelatin	Vacuum desiccator over conc. H_2SO_4	0.93	1.0	93.3
		1.86	2.1	88.3
		2.77	3.4	81.3
		3.66	4.6	79.7
		4.55	6.1	74.6
		5.43	6.9	78.7
				Av. 82.6
Egg white	Vacuum oven 97-98° C.	11.42	14.4	79.0
Dextrin	Vacuum desiccator over conc. H_2SO_4	5.12	5.4	94.7
Gum arabic	Vacuum desiccator over conc. H_2SO_4	1.80	1.9	94.5
		3.55	3.6	98.5
		5.26	5.4	97.3
		6.92	7.2	96.1
		8.55	8.8	97.2
		10.14	10.4	97.5
		11.69	12.0	97.4
		13.21	13.5	97.8
		14.69	15.0	97.9
		16.14	16.4	98.4
		17.56	17.8	98.6
				Av. 97.4

growing season, without introducing serious errors. The maximum deviation from the average relation between dry substance found by refractometer and by oven-drying, among the plant-juice determinations listed in Table V, is that for Banner oats collected July 3. The difference in this case would, in a bound-water determination, cause an error of about 0.012° C. in the apparent excess depression of the freezing point, equivalent to about 0.5% bound water. It is unlikely of course that many errors of such magnitude occurred throughout the work, and even one this large would not be sufficient to affect significantly most of the comparisons made.

HYDROLYTIC ACTION OF PLANT JUICE ON SUCROSE AS AFFECTED BY TIME AND TEMPERATURE

It was pointed out earlier in this paper that the main objection to the use of sucrose in determining the bound water of plant juices was the presence in the latter of invertase, but that in preliminary experiments this factor did not appear to cause serious errors. However, in view of the proposed extensive

use of the method in these investigations, it was deemed advisable at the outset to make a further check of this point. The plan adopted was to estimate the hydrolysis of sucrose not only under the usual working conditions but also under conditions which departed progressively from the normal. This plan makes it possible to show graphically the definite trends which take place with respect to time and temperature of storage of the juice-sucrose mixtures.

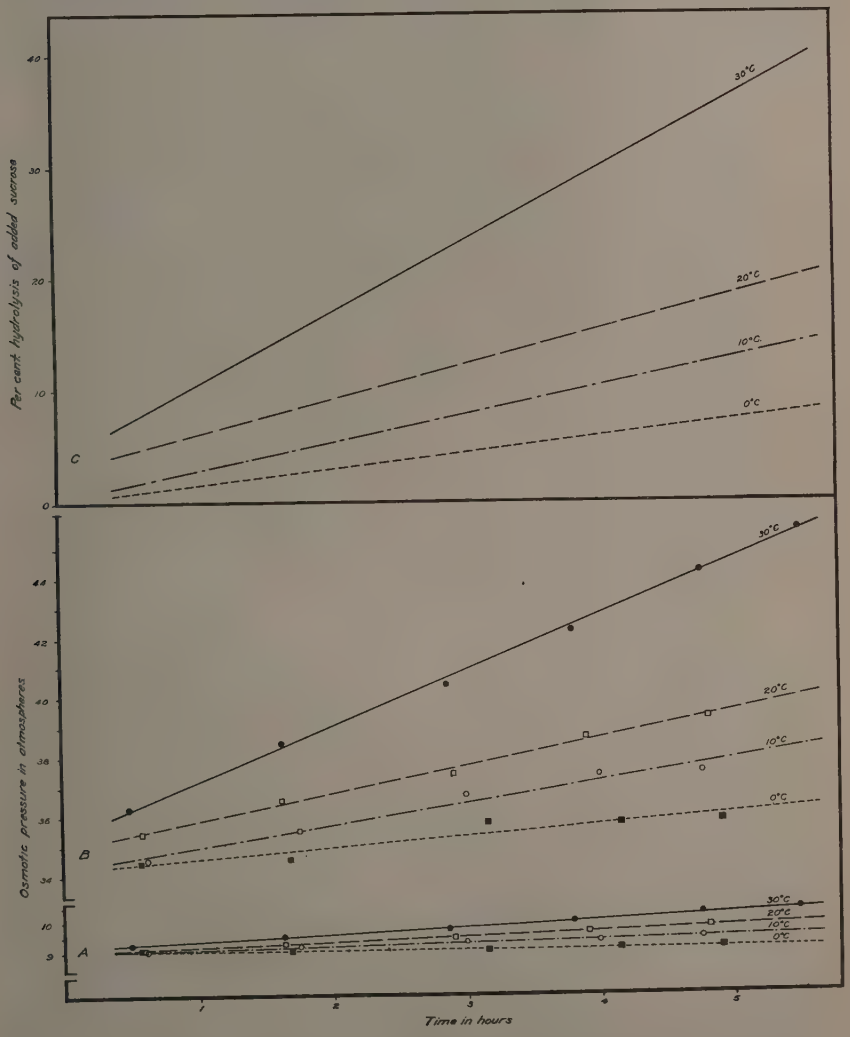

FIG. 5. *Hydrolytic action of wheat-leaf juice on sucrose as affected by time and temperature. A, juice only (autolysis); B, juice+M/1 sucrose; C, rate of hydrolysis of sucrose.*

About 1,200 gm. of juice was expressed from the ground leaf tissue of Marquis wheat seedlings. Into four 150 cc. erlenmeyer flasks were weighed portions of juice, each containing 100 gm. of water. To each flask was added 34.224 gm. of pulverized sucrose, which was dissolved by shaking gently in constant-temperature baths, the first flask being maintained at 0° C., the second at 10° C., the third at 20° C., and the fourth at 30° C. A second portion of juice not containing sucrose was stored in each temperature bath. The freezing-point depression of each of the eight portions of juice was determined at intervals over a period of about five hours.

TABLE VII

HYDROLYTIC ACTION OF WHEAT-PLANT JUICE ON SUCROSE IN RELATION TO TIME AND TEMPERATURE

Temperature	Time in hr.	Juice only		Juice+sucrose		Rate of hydrolysis of sucrose	
		Δ in ° C.	P in atm.	Δ in ° C.	P in atm.	in %	Q_{10}
0° C.	0.57	0.756	9.09	2.875	34.50	0.8	
	1.68	0.750	9.03	2.883	34.59	2.4	
	3.15	0.748	9.00	2.989	35.86	4.7	
	4.15	0.762	9.17	2.981	35.77	5.9	
	4.92	0.759	9.14	2.988	35.85	7.1	—
10° C.	0.62	0.742	8.93	2.879	34.54	1.9	
	1.75	0.761	9.16	2.966	35.57	4.7	
	3.00	0.777	9.34	3.050	36.75	7.8	
	4.00	0.780	9.39	3.108	37.45	10.6	
	4.77	0.784	9.41	3.112	37.50	12.2	1.8
20° C.	0.58	0.758	9.12	2.946	35.50	4.7	
	1.62	0.770	9.27	3.041	36.64	7.8	
	2.90	0.786	9.45	3.106	37.42	11.8	
	3.90	0.800	9.63	3.212	38.70	14.5	
	4.82	0.812	9.77	3.268	39.38	17.7	1.4
30° C.	0.50	0.769	9.25	3.015	36.33	7.1	
	1.63	0.792	9.53	3.197	38.52	14.5	
	2.87	0.817	9.87	3.357	40.45	22.4	
	3.80	0.831	10.01	3.508	42.27	28.3	
	4.77	0.852	10.26	3.672	44.24	34.5	
	5.50	0.864	10.40	3.786	45.62	39.3	2.0

The results are given in Table VII and Fig. 5. The osmotic pressure P is based as usual upon the freezing-point depression. The per cent hydrolysis of the added sucrose, shown under the last heading of the table and in part C of the figure, was calculated from the difference in osmotic pressure of the juice only and the juice+sucrose at any given time as compared with the difference at zero time. The differences were read from the graphs in parts A and B of the figure, and are thus smoothed out. Since the method requires 20 min. shaking of the juice after the sucrose has been added, to ensure complete solution, and since moreover a series of eight samples were handled simultaneously, the differences at zero time had to be found by extrapolation. The experimental data do not appear to justify drawing the corresponding graphs

as other than straight lines, but it must be observed that the lines do not meet at the point indicating zero time. Therefore, although the van't Hoff coefficient Q_{10}, for acceleration of reaction with rise of temperature, has as a matter of interest been added in the last column of the table, it is not desired to emphasize unduly the theoretical importance of these results. They do, however, answer satisfactorily the question for which the experiment was instituted.

No appreciable change took place in the osmotic concentration of the juice itself during five hours storage at 0° C., and the rate of inversion of sucrose at this temperature was comparatively low. With each increase in temperature there was a slightly more pronounced change in the composition of the juice and a decided increase in the rate of inversion of sucrose.

In the bound-water method, the sucrose is dissolved in the juice at 0° C. and the mixture is rarely held more than a half-hour before the duplicate determinations are completed. The maximum error due to hydrolysis of added sucrose would under these conditions not exceed the equivalent of 0.5% bound water. It is to be noted, furthermore, that the bound-water values of all plant juices were determined under the same conditions, and for comparative purposes the error now under consideration would cancel to the extent that corresponding hydrolysis occurred in all cases.

Effect of Storing Leaf Tissue on Osmotic Pressure and Bound-water Content

Although the interval between collecting the leaf tissue in the field and pressing out the fluids was usually short, it naturally varied somewhat with different collections. An experiment was therefore carried out to determine the effect of storage, under the usual conditions maintained in this work, on the content of bound water.

Leaf tissue of Minhardi winter wheat was collected on October 6, 1924, mixed thoroughly to ensure uniformity, and divided into three equal portions. These portions were placed in quart Mason jars packed in ice and removed to the laboratory. The first portion was ground and pressed out immediately, while the second and third portions were stored for one and four hours respectively. In all three cases the osmotic pressure and bound water were determined immediately after pressing out the juice.

TABLE VIII

Effect of storing leaf tissue on osmotic pressure and bound-water content of press juice

Length of storage in hr.	Concentration of juice in %	Δ in ° C.	Osmotic pressure in atm.	Bound water in %
0	16.2	1.380	16.6	9.8
1	16.2	1.380	16.6	9.5
4	16.2	1.382	16.6	8.8

The results presented in Table VIII show that, though there was no change in the osmotic concentration, the bound-water content decreased slightly during storage, even when the tissue was packed in ice. In the present work, the samples in a series were pressed out in the same order in which collected, and the interval between collecting and pressing any given sample seldom exceeded a half-hour. In this time the changes in the properties of the cell fluids would be inappreciable.

Effect of Storing Press Juice at Various Temperatures

The press juice was normally held only a short time after being expressed before the desired determinations were carried out. The nature of this fluid, however, suggested the possibility of rapid changes taking place. The organization of the protoplasmic colloids within the cell must be largely destroyed in the extraction process, which also causes them to become intimately mixed with the vacuolar sap. Consequent changes in hydrogen ion concentration, intermixture of enzymes and substrates, and the general catalytic nature of such a colloidal sol, all make for the establishment of new equilibria.

To ascertain, first, what changes in osmotic pressure and bound water might take place under the conditions of the routine determinations, and, second, what effect on these properties might result from conditions which deviated therefrom progressively with respect to time and temperature of storage, four portions of the same lot of press juice were stored in constant-temperature baths at 0°, 10°, 20°, and 30° C. respectively, over a period of about seven hours. Samples were withdrawn at intervals for the determination of osmotic pressure and bound water. The changes in both these properties are reported in Table IX, and those in the bound-water values are shown again in Fig. 6.

Of the three series indicated in the first column of Table IX as A, B, and C, the latter two are repetitions of the experiment, done because of the irregular nature of the results. In series C an attempt was made to show the trends more accurately by handling press juice held at only one temperature each day, and thus making possible more frequent readings. The absolute magnitude of the figures in different series are therefore not comparable, since differences in date of collection, weather conditions, and variety of plant all affect the composition and concentration of the juice. Series A and B are homogeneous within themselves, but in series C the samples of tissue were collected on four successive days, beginning on a cool moist day after a rainy period and continuing through progressively drier and warmer days. These conditions are reflected especially in the solid contents of the juices and the initial percentages of bound water. In series C, therefore, the results obtained at each temperature must be considered independently.

The osmotic pressures, as would be expected owing to probable hydrolysis of various substances in the juice, in most cases increased gradually during storage, the change being most pronounced at the highest temperature. This point was also dealt with earlier in discussing Table VII. It should be noted

TABLE IX

CHANGES IN OSMOTIC PRESSURE AND BOUND WATER OF PRESS JUICE DURING STORAGE AT 0°, 10°, 20°, AND 30° C.

Material	Temperature of storage in °C.	Time of storage in hr.	△ in ° C.	Osmotic pressure in atm.	Bound water in %
A					
Kubanka wheat collected June 17; solids in juice 6.5%	0	0.0	0.835	10.0	2.7
	0	2.12	0.854	10.3	4.3
	0	5.23	0.846	10.2	3.0
	0	7.85	0.850	10.2	3.2
	10	1.88	0.865	10.4	2.4
	10	4.92	0.864	10.4	2.7
	10	7.58	0.863	10.4	2.4
	20	1.57	0.869	10.5	4.1
	20	4.20	0.902	10.9	2.6
	20	6.72	0.892	10.7	1.5
	30	1.23	0.879	10.6	2.7
	30	3.47	0.910	11.0	0.8
	30	6.32	0.928	11.2	3.0
B					
Caesium wheat collected Sept 1; solids in juice 13.9%	0	0.0	0.983	11.8	4.9
	0	3.17	1.000	12.0	3.4
	0	5.63	1.024	12.3	5.2
	0	7.40	1.055	12.7	6.4
	10	2.98	1.022	12.4	3.4
	10	5.27	1.059	12.7	4.9
	10	6.97	1.086	13.1	6.8
	20	1.45	1.048	12.6	7.7
	20	4.68	1.089	13.1	6.9
	20	6.63	1.146	13.8	11.4
	30	1.15	1.068	12.8	4.4
	30	4.22	1.169	14.1	3.4
	30	6.23	1.208	14.5	7.1
C					
Kubanka wheat collected Sept. 8; solids in juice 7.7%	0	0.0	0.811	9.8	1.0
	0	0.62	0.818	9.9	1.1
	0	1.60	0.830	10.0	0.7
	0	2.27	0.838	10.1	0.4
	0	3.68	0.842	10.1	1.8
	0	4.45	0.847	10.2	2.5
	0	5.42	0.842	10.2	3.4
	0	6.05	0.858	10.3	3.3
	0	6.63	0.864	10.4	3.4
Collected Sept. 9; solids in juice 7.0%	10	0.0	0.756	9.1	1.6
	10	0.72	0.762	9.2	1.7
	10	1.63	0.773	9.3	1.6
	10	2.97	0.777	9.3	1.5
	10	3.65	0.780	9.4	1.8
	10	4.37	0.781	9.4	1.6
	10	5.07	0.788	9.5	1.9
	10	5.57	0.787	9.5	1.8
	10	6.20	0.791	9.5	1.8
Collected Sept. 10; solids in juice 8.6%	20	0.0	0.731	8.8	4.9
	20	1.08	0.883	10.6	1.0
	20	1.93	0.912	11.0	1.0
	20	3.50	0.927	11.2	0.6
	20	4.25	0.938	11.3	0.9
	20	4.90	0.944	11.4	2.0
	20	5.63	0.954	11.5	2.1
	20	6.30	0.958	11.5	2.2
Collected Sept. 11; solids in juice 9.9%	30	0.0	0.889	10.7	5.6
	30	0.77	0.976	11.7	2.7
	30	1.32	1.016	12.3	0.7
	30	1.97	1.049	12.6	1.9
	30	3.52	1.080	13.0	2.0
	30	4.40	1.098	13.2	3.9
	30	5.02	1.114	13.4	3.9
	30	5.60	1.118	13.5	3.8
	30	6.47	1.128	13.6	4.5

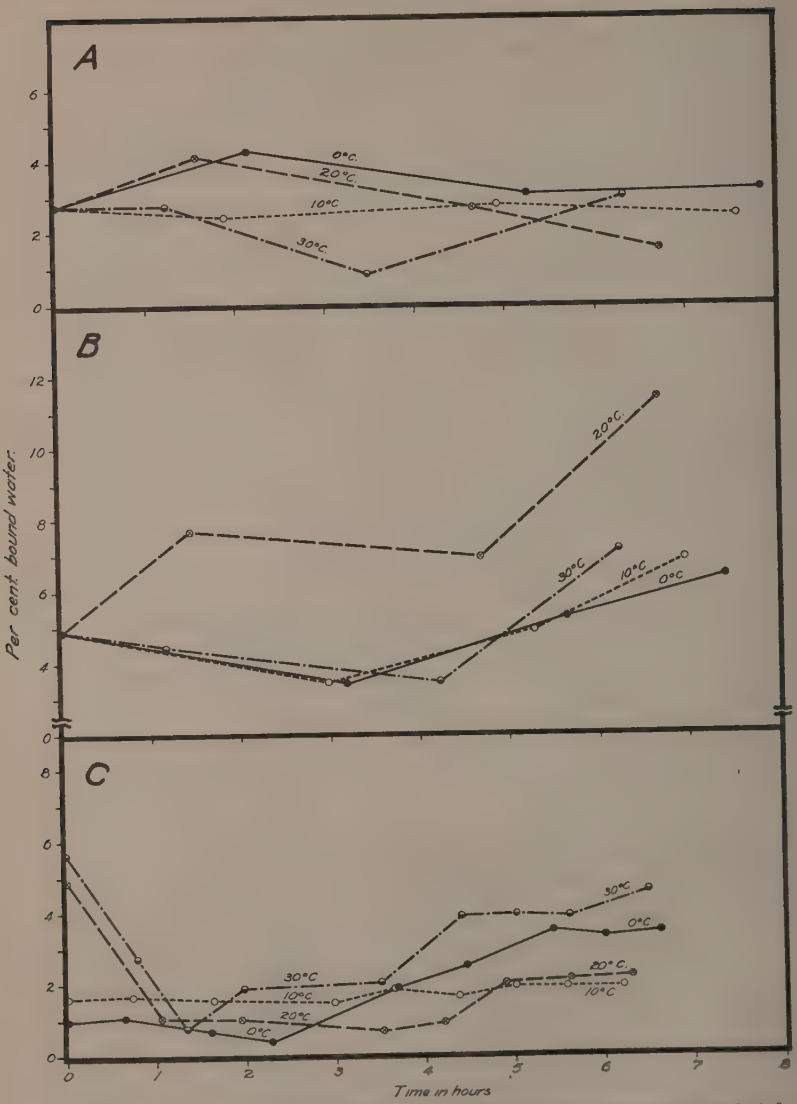

FIG. 6. *Changes in the bound-water values of press juice during storage at 0°, 10°, 20°, and 30° C. Data from series A, B, and C, Table IX.*

here that changes in the osmotic pressure of the juice do not affect the bound-water method, since they are automatically corrected in the procedure. They are, however, important in themselves, since osmotic pressure is one of the physical properties of the juice having a relation to drought resistance, and it is therefore desirable to record values which are correctly representative of those actually obtaining in the unchanged cell fluids. Furthermore, a change

in osmotic pressure is likely to be associated with changes in the physical condition and hydration of the cell colloids, and hence in the bound water. The rate of change in osmotic pressure at 0° C., the temperature used in routine storage, was fortunately not great enough to introduce appreciable errors.

The course of the bound-water values, seen most easily in Fig. 6, varies widely in the different series and is so complicated as to be impossible of explanation without further investigations. The experiment shows the possibility of serious error resulting from holding press juice for unnecessarily long periods even at 0° C. Changes in hydrogen ion concentration are probably partly responsible for the fluctuations in hydration. The points of practical importance which emerge clearly are, first, that the fluctuations were less violent at 0° and 10° than at 20° and 30° C., and, second, that no significant change occurred at 0° C. during the first half-hour, the maximum time required for a bound-water determination. The latter point is seen best in series C, in which the readings were made much more frequently than in the other series.

Summary

By fine grinding of fresh plant tissue and pressing out at low pressure, these operations being carried out close to 0° C., a fluid is obtained which is believed to have substantially the same composition as the original tissue fluids.

The content of dry substance in plant juice may be read approximately by a refractometer equipped with a sugar scale, provided there is applied a suitable correction found experimentally.

The hydrolysis of sucrose added to plant juice increases with time and temperature of storage. The amount of hydrolysis of the sucrose added in bound-water determination is, under the conditions observed, not sufficient to introduce serious errors.

Leaf tissues stored four hours in quart jars packed in ice showed no change in osmotic concentration of the press juice, but the bound-water value fell off appreciably. Press juice stored at 0, 10, 20 and 30° C. increased gradually in osmotic concentration with time and temperature; its bound-water content fluctuated quite widely, especially at the higher temperatures. It is shown that the methods followed in these investigations largely avoid errors due to these causes.

V. Dialysis of Plant Juice and Properties of Dialysed Fluids

During the seasons of 1924 and 1925, the studies were limited to the properties of the tissue fluids as expressed from the leaves. In 1926, they were extended to include separation of the colloidal constituents by dialysis. It was hoped to obtain in this way certain additional data, more particularly on the per cent hydration of the colloids, or as it is expressed in this paper, the water bound per gram of colloid. This was shown earlier (Section III) to vary considerably with different colloidal substances, and was presumed to be related to the "quality" factor in determining the water-binding capacity of any sol.

From the data tabulated later (Section VI) for 1924 and 1925, the average hydration of the total dry substance in the plant juices may readily be derived, but we hesitated to add such data to the tables because of the probable wide variation in ratio of colloids to crystalloids and the misleading impression which such unequally weighted data might give in regard to the water-binding capacity of the colloids.

The extension of the programme to include dialysis necessitated the development of suitable methods and an examination of the effects of dialysis on the properties of the press juice. The experiments involved in this preliminary work are reported in this section.

Method of Dialysis

Preparation of Sacs

The method developed for the preparation of collodion dialyser sacs of suitable and uniform permeability will first be described. A satisfactory solution of collodion was prepared by dissolving 40 gm. "Union cotton"* in 750 cc. anhydrous ether and 250 cc. 95% ethyl alcohol. Dialyser sacs of 100 cc. capacity were made with the help of the specially constructed apparatus shown in Fig. 7. This consists essentially of a motor-driven, slowly rotating shaft, bearing at one end a wooden block bored to receive snugly the bottom end of a 100 cc. test tube. A glass tube connected with a water pump, and adjustable with respect to depth of insertion into the test tube, is used to aspirate vapors.

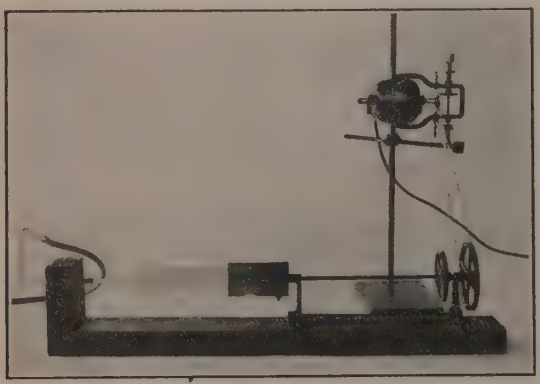

Fig. 7. *Apparatus for preparing dialyser sacs.*

The collodion is poured into the test tube, decanted, and allowed to drain for 30 sec., during which period the tube is gently rotated at an angle of about 60°; then the tube is inserted in the machine and rotated slowly for 2.5 min., while alcohol and ether vapors are aspirated from the interior. The tube is again filled with collodion, decanted, allowed to drain for 30 sec., and rotated in the machine for three minutes. It is next removed from the machine and the top of the sac reinforced by applying, with a stirring rod, a band of collodion a half-inch in width around the top inside. Then the tube is replaced in the machine, the aspirator intake adjusted to extend about an inch beyond the top of the sac, and rotated for two minutes. Finally the tube is removed from the machine, filled with water, the top loosened from the glass by rolling with the thumb, and a jet of water directed down between the tube and sac. If

* *Procured from Chas. Cooper & Co., New York City.*

necessary the sac may be freed from the tube by inserting a small glass rod specially tapered and with an oval knob at the end. Usually, however, the sac is readily freed from the glass by the gentle force of the stream of water.

The sac thus prepared is then immersed in a 1% gelatin sol for one hour, followed by hardening in 1% formaldehyde solution for another hour. It is then stored in distilled water saturated with toluene, until used.

Sacs prepared in this way have been found to be satisfactory and fairly uniform with respect to permeability. In the initial experiments three applications of collodion were used, but the sacs were found to be too impermeable, as indicated by the development of high osmotic pressures during the initial period of dialysis. The pressure thus developed made it difficult to prevent the stoppers being forced out, with a consequent dilution of the fluids.

The effect of the shape and size of dialyser sacs was also investigated. The 100 cc. sacs were about as large as could conveniently be stoppered in the cylindrical form used. Globe-shaped dialysers seemed to offer certain advantages where large quantities of fluids were to be dialysed. In Table X are given the results of an experiment in which the press juice of emmer plants was dialysed 42.5 hr. in both cylindrical and globular sacs.

TABLE X

EFFECT OF SHAPE AND SIZE OF DIALYSER SAC ON EFFICIENCY OF DIALYSIS

Shape and capacity of dialyser sac	Conc'n by drying in %	Osmotic pressure in atm.	Conductivity at 25° C. $K \times 10^3$	H ion conc'n pH	Gold number	Bound water in %
Fresh juice (undialysed)	6.91	8.09	10.10	6.13	0.09	1.7
Cylindrical sac, volume 100 cc.	3.06	0.14	0.37	6.22	0.46	1.9
Globular sac, volume 250 cc.	3.67	1.02	1.35	6.21	0.66	2.4

Dialysis was much more efficient with the cylindrical than with the globular sacs, as shown by the concentration (apparent colloidal content), osmotic pressure, and electrical conductivity at the end of the period. The experiment does not enable us to say what part of the difference was due to shape and what part to size of sac, but it is safe to assume that the larger diffusion area of the cylindrical sacs, in relation to their volume, is mainly responsible for their greater efficiency. Our reason for trying globular sacs was the possibility they offered of handling larger volumes of juice. The experiment showed that their adoption would involve an extension of time of dialysis, a procedure which is inadvisable with biological fluids.

The gold numbers of the fresh and dialysed juice indicate a deterioration in protective quality of colloids during dialysis, more pronounced in the globular sacs. An increase in gold number, it may be stated here, almost invariably takes place when plant juice is dialysed. The direction of change in the bound-water values in the present experiment, however, is abnormal, as these usually

decrease on dialysis. It is interesting to note that the osmotic pressure and conductivity of the juice dialysed in cylindrical sacs are well within the range found in various prepared sols (Tables II and III).

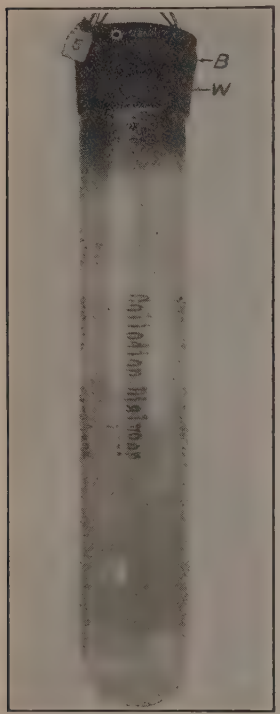

FIG. 8. *Dialyser sac ready for use.*

Dialysis

The press juice is poured into the dialyser sac (Fig. 8), which is closed with a rubber stopper and a rubber band *B*, the latter a section of gooch tubing about a half-inch long drawn over the stopper and enclosing the margin of the sac. The stopper is secured in place by twisting a piece of copper wire *W* tightly around the rubber band. The sacs are completely filled with juice (to prevent changes in volume), but when closed as described will readily withstand the initial osmotic pressure developed when immersed in a water bath.

To carry out the dialysis conveniently and under standard conditions, the apparatus shown in Fig. 9 was devised. A note already published (41) on this apparatus will be reproduced here in part.

The supply of distilled water to the dialyser sacs immersed in the diffusion vessel *V* is provided by an ordinary still kept running during dialysis. The water enters at *W*, dropping through a layer of toluene *T* floated on the surface of the water in *R*, a carboy. *R* and *R'* act as reservoirs. The dialysing system is enclosed in *F*, an electrically refrigerated box in which the cold temperature is automatically held within fairly close limits. The water is cooled by passing through *C*, a coil of block-tin tubing. The bottle *B* is provided with a siphon adjustable for height at *A*. This siphon is of relatively small bore and requires three or four minutes to empty the contents of *B* into the diffusion vessel *V*. The latter has a large-bore siphon, which empties the contents of *V* into the drain *D* in less than one minute. The inner end of the large siphon drops into a small sump in the bottom of *V*, thus ensuring the complete emptying of this vessel.

The diffusion vessel *V* is filled with water to the bend in its siphon. When *B* begins to discharge into *V*, the large siphon of the latter vessel is automatically brought into operation. The quick draining of *V* breaks the action of its siphon a few moments later, allowing the vessel to be refilled at once by the remaining discharge from *B*. The frequency of water changes in the diffusion vessel is regulated by the stopcock *S*, and also by drawing out to a small bore the end of the supply tube entering *B*. In this work the system was adjusted to change the water once every hour, and continued to do so without further attention as long as desired. Any change in the number or

volume of the tubes in the diffusion vessel would of course necessitate a change in the adjustment of the upper siphon at *A*. In practice we found it more convenient to use a constant number of twelve tubes, simply filling with water those not required in any particular experiment.

The cooling system was adjusted to maintain the water in the diffusion vessel at 3° C., since in other experiments in this laboratory it has been found that less coagulation of press-juice colloids takes place at this temperature than at 0° C.

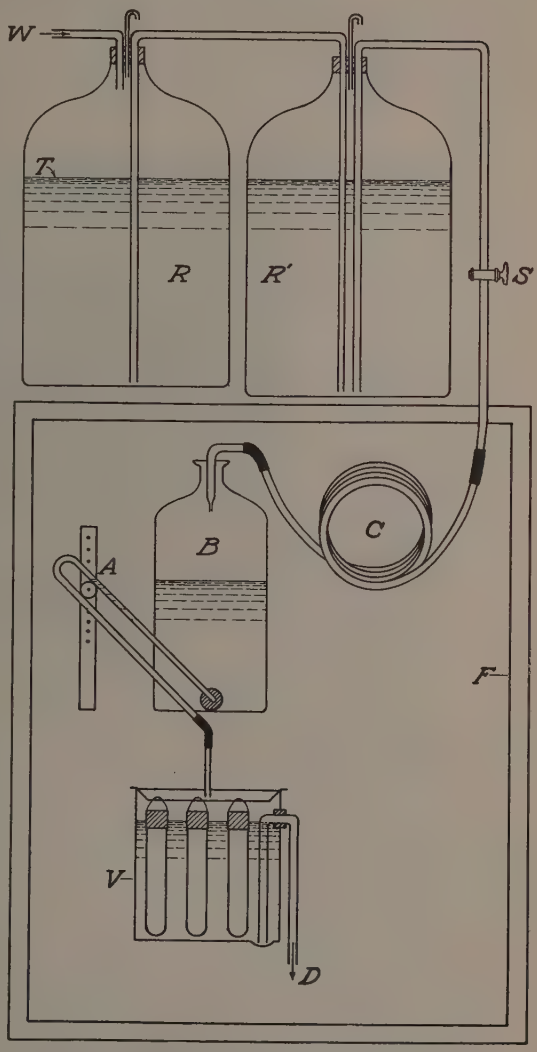

FIG. 9. *Apparatus for continuous dialysis at low temperature.*

PROGRESSIVE CHANGES IN PROPERTIES OF PRESS JUICE DURING DIALYSIS

To determine the effect of dialysis on the properties of plant-tissue fluids, two experiments were carried out, using the press juice of common emmer and Marquis wheat plants respectively. In the first case only four tubes were dialysed, but in the second case the dialysing apparatus was used to capacity, being filled with 12 tubes. These tubes were withdrawn one at a time, at intervals over a period of about 90 hr., and the properties of the contents determined. The values found, together with those for the original juice, are shown in Table XI. The data in part B of this table, for the more extensive experiment with Marquis wheat, have been used to construct Fig. 10, in which all values are calculated and plotted as percentages of the values found with fresh juice.

The concentration of both the fresh and dialysed juice was determined by (a) refractometer, corrected 17% (Table V), and (b) by first drying in a venti-

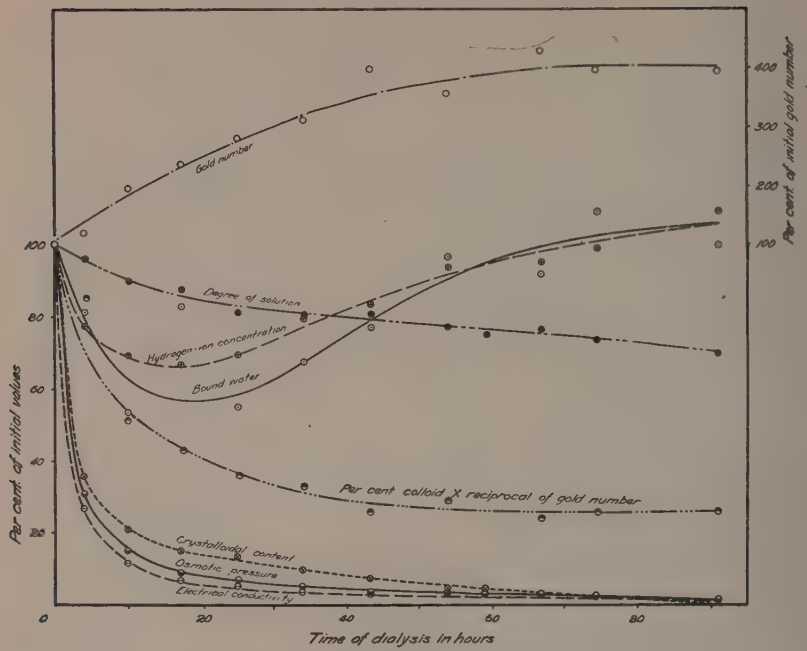

FIG. 10. *Relative changes in properties of press juice of Marquis wheat leaves during the progress of dialysis.*

lated oven at 60° C. for 24 hr., and then in a vacuum oven at 96° C. for 48 hr. The two sets of figures, given in the third and fourth columns of the table, show a satisfactory agreement for the fresh juice, but diverge on dialysis, especially in the experiment with Marquis juice, in which the divergence increases with time of dialysis. This is taken to indicate that there is a progressive coagulation or precipitation of colloids during dialysis, the resulting particles in suspension failing to affect the refractive index. The relation between the two sets of values has been made the basis for calculating the "degree of solution" (the opposite of degree of coagulation), shown graphically in Fig. 10. The final concentration found by drying (shown in italics) is taken as the colloidal content, and the difference between this and the concentration at any earlier time is taken as the crystalloidal content at that time.

The difference in degree of coagulation of the emmer and Marquis juices during the dialysis is associated with a radically different course of bound-water values. Both of these differences are presumably due in turn to differences in the properties of the fresh juices. It may be noted that the emmer had a lower absolute content of colloids and a lower ratio of colloids to crystalloids, this ratio having a value of 0.64 as compared with a value of 1.0 in the Marquis juice. On the other hand, the emmer had a higher absolute concentration of crystalloids, but a lower conductivity, more of the crystalloids being apparently in the form of sugars. The emmer juice was also appreciably less

TABLE XI

PROGRESSIVE CHANGES IN PROPERTIES OF WHEAT-LEAF PRESS JUICE DURING DIALYSIS

Material	Time of dialysis in hr.	Conc'n by refract. in %	Conc'n by drying in %	Crystalloidal content in %	Osmotic pressure in atm.	Conductivity at 25° C. $K\times10^3$	H ion conc'n pH	Gold number	Bound water in %	Bound water per gm. colloid in gm.	Percent colloid / gold number
A	Fresh juice	7.05	6.91	4.22	8.09	10.10	6.13	0.09	1.7	0.60	29.9
Common emmer collected	19.6	2.49	3.06	0.37	0.12	0.40	6.09	0.13	2.0	0.74	20.7
June 8	42.5	2.49	2.82	0.13	0.12	0.37	6.22	0.40	2.2	0.78	6.7
	67.5	2.32	2.78	0.09	0.12	0.27	6.24	0.40	1.3	0.46	6.7
	87.1	2.32	*2.69*	0.00	0.12	0.26	6.20	0.40	1.4	0.49	6.7
B	Fresh juice	8.13	8.08	4.03	8.41	14.88	5.88	0.09	2.8	0.63	43.5
Marquis wheat collected	4.0	5.31	5.50	1.45	2.58	3.97	5.99	0.11	2.2	0.52	36.8
Aug. 31	10.0	4.40	4.90	0.85	1.26	1.75	6.05	0.18	1.5	0.35	22.5
	17.2	4.07	4.66	0.61	0.71	1.02	6.05	0.22	2.3	0.54	18.4
	25.0	3.74	4.60	0.55	0.56	0.78	6.03	0.26	1.5	0.36	15.6
	34.0	3.57	4.44	0.39	0.42	0.56	5.98	0.29	1.9	0.46	14.0
	43.3	3.49	4.34	0.29	0.32	0.52	5.96	0.37	2.1	0.50	10.9
	54.0	3.24	4.23	0.18	0.28	0.42	5.91	0.33	2.7	0.63	12.3
	59.2	3.15	4.22	0.17	0.26	—	—	—	—	—	—
	66.7	3.15	4.14	0.09	0.24	0.37	5.90	0.40	2.5	0.60	10.1
	74.3	2.99	4.09	0.04	0.16	0.32	5.88	0.37	3.0	0.71	10.9
	90.8	2.82	*4.04*	−0.01*	0.14	0.25	5.85	0.37	2.8	0.67	10.9
	90.8	2.82	*4.06*	+0.01*	0.17	0.26	5.83	0.37	2.7	0.63	10.9

**Taken as zero.*

TABLE XII

EFFECT OF DIALYSIS ON 1% SODIUM CHLORIDE SOLUTION* ON PROPERTIES OF WHEAT-LEAF PRESS JUICE

Material	Time of dialysis in hr.	Conc'n by refract. in %	Conc'n by drying in %	Crystalloidal content in %	Osmotic pressure in atm.	Conductivity at 25° C. $K\times10^3$	H ion conc'n pH	Gold number	Bound water in %	Bound water per gm. colloid in gm.	Percent colloid / gold number
Common emmer; colloidal	Fresh juice	8.63	8.43	4.96	11.1	15.3	6.22	0.08	2.7	0.71	43.4
content 3.47%	22.5	5.31	5.38	1.91	9.8	19.3	6.09	0.09	2.1	0.57	38.6
	42.3	4.32	4.74	1.27	7.8	15.7	6.09	0.09	2.6	0.71	38.6
	69.2	3.82	4.42	0.95	7.0	14.3	6.09	0.19	2.1	0.58	18.3
Marquis wheat; colloidal	Fresh juice	7.14	7.35	4.38	10.4	15.7	6.20	0.10	1.6	0.50	29.7
content 2.97%	22.5	4.48	4.74	1.77	9.6	18.9	6.06	0.11	1.8	0.58	27.0
	42.3	3.57	4.20	1.23	7.8	16.0	6.03	0.13	2.0	0.66	22.8
	69.2	2.82	3.93	0.96	7.1	14.6	6.02	0.22	2.0	0.64	13.5

**1.0% NaCl solution: $P=6.17$ atm.; $K\times10^3=14.57$; pH = 7.80.*

acid in reaction than the Marquis juice. These are obvious differences, which may be factors in the behavior of the juice during dialysis, but are not suggested as a complete explanation. Moreover, they are not to be taken as characteristic differences between these varieties: for one thing, the collections were made at different dates. It is clear, however, from a comparison of the two parts of Table XI, that we must be prepared for considerable variability in the effect of dialysis on the hydration of colloidal constitutents in different samples of juice.

In points other than that just mentioned, the behavior of the two varieties was essentially similar, and may be seen best in Fig. 10. The curves for crystalloidal content, osmotic pressure, and electrical conductivity show as would be expected a high initial rate of dialysis, progressively decreasing with time. Since each point on the curves is the result of a determination on the contents of a separate dialyser sac, the smoothness of the curves is evidence of the uniform permeability of these sacs. This is further shown by the close correspondence in all properties of the contents of the last two sacs (see table, not figure), which were taken out at the same time.

The values for per cent colloid multiplied by the reciprocal of the gold number represent an attempt to obtain a figure which takes account of both quantity and quality of colloid. The curve for this value drops quite rapidly at first and subsequently flattens out. The falling-off would be expected from the apparent coagulation during dialysis, as shown by the curves for degree of solution and gold number. These last two curves are nearly reciprocal in shape, indicating that the protective value of the colloidal fraction remaining in solution was not greatly different from that of the original whole amount.

On the other hand, there is no simple relation between the foregoing properties and hydration of colloids as shown by per cent bound water and (in the table only) bound water per gram of colloid. Hydration is affected by hydrogen ion concentration and probably by other factors as well. The curve for hydrogen ion concentration (shown in the figure as cH, not pH) first falls and then rises again to a value slightly above the original, its course presumably being governed by the relative diffusion rates of various salts differing in reaction. The bound-water curve, though less satisfactory than any of the others because of irregular scattering of the points, does seem in a general way to parallel that for hydrogen ion concentration.

As a result of these and other experiments, a time of about 48 hr. was adopted for routine dialyses. This represents a compromise between the desire to effect complete removal of the crystalloids and the desire to avoid excessive alteration of the colloids. More important than this technical point, however, is the light the experiments throw on the behavior of plant juice during dialysis and the interrelations of the various properties under investigation.

Dialysis with Solution of Common Salt

The foregoing experiments showed that there was a tendency for press-juice colloids to be coagulated upon removal of crystalloids by dialysis. Analogous behavior had been observed in egg white (Table IV, Fig. 4), the globulin

fraction of which was soluble in the natural medium, but was thrown out of solution by the addition of water. An experiment was therefore carried out to see whether the colloidal state of the solids might be preserved by dialysis with a salt solution. This would of course defeat the purpose of isolating the colloids, but would at least secure them in a solution of approximately constant composition with respect to crystalloids.

A 1% solution of commercial salt from Fort McMurray, Alberta, was used. This solution had an electrical conductivity close to that of the wheat-leaf tissue fluids being studied, and this seemed the best basis on which to adjust the concentration. The press juice of common emmer and Marquis wheat was dialysed in this solution, both varieties at the same time, tubes being withdrawn for examination at intervals during about 69 hr. The results are presented in Table XII.

The crystalloidal content, shown in the fifth column of the table, is expressed as a percentage by weight. Consequently, the last reading for each variety is not exactly 1.0, owing to the difference in specific gravity of the juice and the salt solution. For the same reason the last reading in the preceding column, for concentration by drying, is not exactly equal to the colloidal content plus 1.0. The colloidal content has been given for convenience in the first column of the table.

A general survey of the table reveals the same tendencies observed in the preceding experiments. Although the changes in the colloids were not so marked as when dialysed in distilled water, the original properties were not preserved by the salt. Again we see on comparing the concentration determined by refractive index with that determined by drying, evidence of progressive precipitation, as before more pronounced in the Marquis juice. It is interesting to note that both varieties increased slightly in hydrogen ion concentration during dialysis, in spite of the fact that the salt solution in which the sacs were immersed was decidedly more alkaline than the juice—possibly an example of Donnan equilibrium.

The experiment showed the impracticability of preserving the original colloidal state by the use of common salt, and pending further investigation the method of dialysis in distilled water was retained.

Effect of Hydrogen Ion Concentration on Properties of Fresh and Dialysed Press Juice

The important effect of hydrogen ion concentration on the hydration of colloids is well known, and in the results of the foregoing experiments indications were not lacking that the colloidal constituents of plant juice are no exception to the rule. To throw additional light on this point, experiments were carried out in which the hydrogen ion concentration of fresh and dialysed press juice was altered by the addition of various substances.

Lactic acid and sodium carbonate were the substances chiefly used, and at the outset consideration was given to the possibility of the sucrose used in the bound-water determinations being hydrolysed or otherwise affected by their

addition. The freezing-point depression of a molar solution of sucrose was determined for comparison in distilled water and in dilute solutions of lactic acid, under conditions similar to those observed in determining bound water. The effect of the alkaline salt, sodium carbonate, was determined in like manner for solutions containing various concentrations of salt but constant weights of water and sucrose. The results are presented in Table XIII, and again for sodium carbonate in Fig. 11.

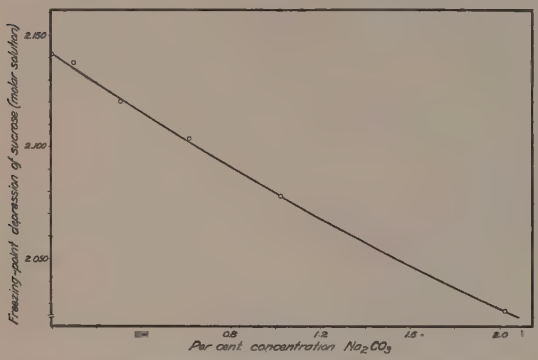

FIG. 11. *Freezing-point depression of molar sucrose in sodium carbonate solutions of various concentrations.*

The stock solution of lactic acid had a concentration of 85%. Two solutions of about 0.5% concentration were made up, and although the apparent freezing-point depression of sucrose differed slightly in the two cases, the average value is almost exactly that of sucrose in distilled water, or 2.124 as compared with 2.125° C. In the second case, a rather extended series of freezing-point determinations was made with three separate portions of the acid-sucrose solution (designated a, b and c in the table), and no progressive hydrolysis could be detected during the considerable time required for the experiment. It was therefore concluded that at 0° C. the rate of hydrolysis was too low to affect appreciably the accuracy of bound-water determinations.

On the other hand, it was found that sodium carbonate in a concentration of only 0.1% caused already a measurable decrease in the freezing-point depression of the sucrose. The experiment was therefore extended to include a series of concentrations up to about 2%. There proved to be an almost linear relation between salt concentration and decrease in the apparent freezing-point depression of the sucrose. The cause for this has not been investigated, though it may be that this salt reduces the hydration of the sucrose, or that the sodium ions react with the sugar to form a salt, or even that the alkali brings about a certain amount of polymerization. Whatever the explanation, the curve in Fig. 11 has been made the basis for correcting the bound-water values in the following experiments in which sodium carbonate was added.

Two experiments were carried out in which the hydrogen ion concentration of fresh and dialysed press juice of Marquis wheat leaves was increased by the addition of lactic acid and decreased by the addition of sodium carbonate, both reagents being added in short series of increasing concentrations. In Table XIV are presented the results of both experiments, designated A and B respectively. The relation of bound-water content to hydrogen ion concentration, found in the more extensive experiment B, is shown graphically in Fig. 12.

TABLE XIII

EFFECT OF LACTIC ACID AND SODIUM CARBONATE ON FREEZING-POINT DEPRESSION OF MOLAR SUCROSE

Sample	No. of determinations	Difference in maximum and minimum values in ° C.	Δ solution (mean) in ° C.	Δ sucrose (apparent) in ° C.
Lactic acid				
100 gm. water +34.224 gm. sucrose	8	0.003	2.125	—
200 cc. water +1 cc. lactic acid	6	0.003	0.114	—
Do. + sucrose	6	0.004	2.247	2.133
200 cc. water +1 cc. lactic acid	12	0.004	0.115	—
Do. + sucrose (a)	8	0.006	2.238	2.123
(b)	4	0.005	2.232	2.117
(c)	6	0.002	2.240	2.125
				Av. 2.124
Sodium carbonate				
100 gm. water +34.224 gm. sucrose	20	0.006	2.141*	—
100 gm. water +0.1 gm. Na_2CO_3	5	0.001	0.054	—
Do. +sucrose	9	0.019	2.192	2.138
100 gm. water +0.3097 gm. Na_2CO_3	5	0.004	0.158	—
Do. +sucrose	9	0.016	2.276	2.118
100 gm. water +0.6193 gm. Na_2CO_3	4	0.002	2.295	—
Do. +sucrose	5	0.020	2.398	2.103
100 gm. water +1.0322 gm. Na_2CO_3	5	0.003	0.455	—
Do. +sucrose	10	0.024	2.533	2.078
100 gm. water +2.0644 gm. Na_2CO_3	5	0.003	0.845	—
Do. +sucrose	7	0.010	2.871	2.026

**Sucrose used from different stock to that used in lactic acid experiments.*

A comparison of the apparent concentration read with the refractometer and that found by drying the juice indicates that the lactic acid caused in the fresh juice a precipitation of the colloids, increasing with concentration of the acid. This conclusion is borne out by the corresponding increase in the gold number (Experiment B). It is true that the gold number must be interpreted with caution in experiments in which electrolytes are added, since the method for its determination involves the interaction of electrical charges. However, it will be observed that in the dialysed juice, in which the colloids were peptized rather than precipitated by the lactic acid, the gold number shows a corres-

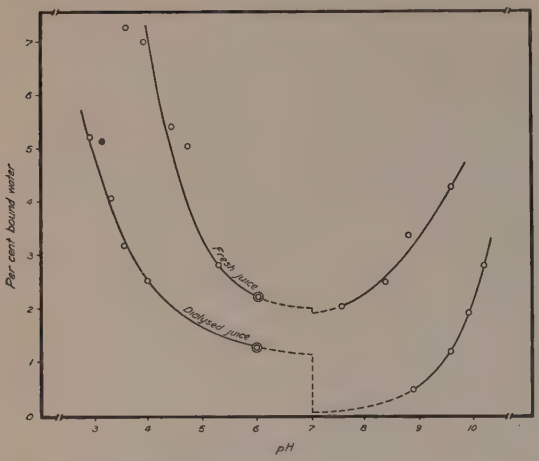

FIG. 12. *Effect of lactic acid and sodium carbonate on hydrogen ion concentration and bound water in fresh and dialysed press juice of Marquis wheat leaves. Reaction of untreated juice shown by double circles.*

ponding decrease. The colloids in the untreated dialysed juice were in both experiments apparently in a state of partial precipitation. The opposite effects of the lactic acid on the state of dispersion of colloids in the dialysed and undialysed juice show the importance of the natural crystalloids in controlling colloidal reactions.

The addition of sodium carbonate, though it changed the hydrogen ion concentration even more than the lactic acid, and in Experiment A brought about a greater increase in bound water, caused no precipitation of the fresh juice. In the dialysed juice, like the lactic acid, its effect was one of peptization.

The relation of hydrogen ion concentration and hydration of the colloids may be seen best in Fig. 12. The per cent bound water reaches a minimum at the neutral point or, no doubt more exactly, at the isoelectric point of the plant colloids. From this point hydration increases with either an increase or decrease in hydrogen ion concentration. This is in accordance with the well-known behavior of hydrophilic colloids in general.

The points plotted on the graph seem to indicate that the curves do not meet at the isoelectric point (an interpretation we have accepted) but show an initial depression of hydration upon the addition of sodium carbonate. This again is in accordance with the usual effect of salts on the swelling of colloids. The location of the points on the alkaline side of the curves thus represents a compromise between the effects of two opposing factors, salt concentration and hydrogen ion concentration. The salt effect is much less obvious in the fresh juice, in which it is masked by that of the natural salt already present. An analogous distinction between the fresh and dialysed juice is seen in the much greater change in hydrogen ion concentration caused in the latter by the initial additions of lactic acid and sodium carbonate; the buffer effects of the natural electrolytes were absent in the dialysed juice.

The concave shape of the curves, with a comparatively broad zone of low hydration in the region of the isoelectric point, is also in harmony with the observations of other workers on plant colloids. Uhlela (51) found the swelling curves of succulent cactus tissue to be of this shape.

The lower level of hydration in dialysed as compared with fresh juice is in line with that generally found throughout these investigations, and seems too

TABLE XIV

EFFECT OF HYDROGEN ION CONCENTRATION ON COLLOIDAL PROPERTIES OF FRESH AND DIALYSED PRESS JUICE OF MARQUIS WHEAT LEAVES

Sample	Conc'n by refract. in %	Conc'n by drying* in %	H ion conc'n pH	Gold number	Bound water in %	Bound water per gm. colloid in gm.	Per cent colloid / gold number
A†*Fresh juice*							
100 cc.+2.0 gm. Na_2CO_3	10.9	11.0	9.49	—	4.4	0.96	—
100 cc.+1.0 gm. Na_2CO_3	10.2	10.1	8.79	—	2.7	0.60	—
Fresh juice only	9.6	9.2	6.91	—	1.3	0.26	—
100 cc.+1.0 cc. lactic acid	6.6	9.9	4.40	—	2.6	0.58	—
100 cc.+2.0 cc. lactic acid	8.2	10.6	3.87	—	3.2	0.70	—
Dialysed juice							
Dialysed juice only	2.4	4.1	6.22	0.70	1.3	0.31	5.8
100 cc.+1.0 cc. lactic acid	—	4.9	3.05	0.15	4.5	1.06	27.1
100 cc.+2.0 cc. lactic acid	—	5.7	2.74	0.11	5.3	1.23	36.8
B†*Fresh juice*							
100 cc.+2.0 gm. Na_2CO_3	13.2	13.3	9.57	—	4.2	0.66	—
100 cc.+1.0 gm. Na_2CO_3	12.4	12.5	8.78	0.18	3.4	0.53	30.7
100 cc.+0.6 gm. Na_2CO_3	12.2	12.2	8.36	0.20	2.5	0.39	27.6
100 cc.+0.3 gm. Na_2CO_3	12.1	11.9	7.53	0.25	2.0	0.32	22.1
Fresh juice only	11.8	11.7	6.00	0.25	2.2	0.35	22.1
100 cc.+0.3 cc. lactic acid	11.8	11.9	5.26	0.30	2.8	0.45	18.4
100 cc.+0.6 cc. lactic acid	10.1	12.0	4.70	0.35	5.0	0.80	15.8
100 cc.+1.0 cc. lactic acid	9.5	12.4	4.38	0.45	5.4	0.85	12.3
100 cc.+2.0 cc. lactic acid	9.1	13.1	3.90	—	7.0	1.10	—
100 cc.+3.0 cc. lactic acid	9.5	13.7	3.56	—	7.2	1.12	—
Dialysed juice							
100 cc.+2.0 gm. Na_2CO_3	7.9	7.3	10.14	—	2.8	0.46	—
100 cc.+1.0 gm. Na_2CO_3	6.4	6.4	9.87	—	1.9	0.32	—
100 cc.+0.6 gm. Na_2CO_3	5.8	6.1	9.54	—	1.1	0.20	—
100 cc.+0.3 gm. Na_2CO_3	5.6	5.8	8.84	0.05	0.4	0.08	110.6
Dialysed juice only	4.5	5.5	5.95	0.46	1.3	0.22	12.0
100 cc.+0.3 cc. lactic acid	5.1	5.8	3.94	0.25	2.5	0.43	22.1
100 cc.+0.6 cc. lactic acid	5.6	6.0	3.50	0.20	3.1	0.54	27.6
100 cc.+1.0 cc. lactic acid	6.2	6.3	3.26	0.10	4.0	0.69	55.3
100 cc.+2.0 cc. lactic acid	6.8	7.0	2.86	0.04	5.2	0.87	138.2

**Concentration of fresh and dialysed juice determined by oven-drying; other values in this column calculated.*

†*Sample A, fresh juice:* $P=10.2$ *atm.,* $K\times10^3=16.4$*; dialysed juice:* $P=0.20$ *atm.,* $K\times10^3=0.36$.

Sample B, fresh juice: $P=11.5$ *atm.,* $K\times10^3=14.2$*; dialysed juice:* $P=0.53$ *atm.,* $K\times10^3=0.48$.

great to be accounted for by the hydration of crystalloidal substances. The partial precipitation of the colloids upon removal of the natural electrolytes has already been noted, but cannot be regarded as a complete explanation. It is difficult to reconcile, for example, with the association of an apparent precipitation and simultaneous increase in bound water on the addition of lactic acid to fresh juice in the present experiment, unless we are to assume that the increased hydration of the colloids remaining in solution was so great as to more than offset any decrease in hydration by precipitation. It must also be remembered that the experiments with prepared sols (Table II) indicated that the state of dispersion might have very little to do with hydration. The reason for the greater hydration of press-juice colloids in the presence of the natural crystalloids must await further investigation. It seems quite possible that sugars, which in other work (37) have been shown to be protective substances, may prove to be equally important with hydrogen ion concentration.

To check more fully the salt effect noted above, a further experiment was carried out, in which the alkaline salt, Na_2CO_3, the neutral salt, NaCl, and the acid salt, KH_2PO_4, were added in equimolar weights to equal portions of the dialysed press juice of emmer leaves. The juice had been dialysed 64 hr. The results are given in Table XV.

TABLE XV

EFFECT OF ALKALINE, NEUTRAL AND ACID SALTS ON COLLOIDAL PROPERTIES OF DIALYSED PRESS JUICE OF EMMER LEAVES

Sample	Conc'n by refract. in %	Conc'n by drying* in %	H ion conc'n pH	Gold number	Bound water in %	Bound water per gm. colloid in gm.	Per cent colloid / gold number
Dialysed juice only†	5.6	6.6	6.11	0.25	2.3	0.32	26.3
75 cc.+1.50 gm. Na_2CO_3	8.5	8.2	10.05	0.05	2.5	0.35	131.4
75 cc.+0.8278 gm. NaCl	7.5	7.6	6.05	0.25	2.0	0.28	26.3
75 cc.+1.9273 gm. KH_2PO_4	5.7	8.7	5.22	0.35	2.9	0.40	18.8

**Concentration of dialysed juice determined by oven-drying; other values in this column calculated.*
†Dialysed juice, untreated: $P = 0.94$ atm., $K \times 10^3 = 0.69$.

In regard to the effect on state of solution, as shown by refractive index and gold number, it will be seen that sodium carbonate as before dispersed the partially precipitated dialysed colloids. Sodium chloride had little effect, but the acid phosphate caused a slight increase in precipitation. Again, however, this change in degree of dispersion seemed on the acid side largely independent of hydration; the bound water was increased by acid potassium phosphate.

The changes in bound water, though less marked than in the preceding experiments, still followed the course of the hydrogen ion concentration. The salt effect was demonstrated by, first, the lower hydration in the presence of the neutral salt as compared with that in the untreated juice, and, second, the proportionately lesser increase in hydration in the presence of the acid salt as compared with that caused by a similar change in hydrogen ion concentration

brought about in the previous experiment by the addition of lactic acid.

The partial precipitation of colloids on dialysis, which has been frequently referred to, is not usually observable to the eye, but has been judged mainly by the change in refractive index, and to a limited extent by the gold number. The suspended particles are not large enough to settle perceptibly under the ordinary conditions of our experiments. In one special experiment, however, we had recourse to centrifuging to determine the state of dispersion of the colloids in dialysed juice, with and without the addition of lactic acid and sodium carbonate. Both of these substances had been found to peptize the solids in dialysed juice, as judged by the above criteria.

TABLE XVI

EFFECT OF SODIUM CARBONATE AND LACTIC ACID ON STATE OF DISPERSION OF COLLOIDS IN DIALYSED PRESS JUICE OF EMMER LEAVES

Sample	H ion conc'n pH	Solids in 5 cc. juice in gm.	Solids thrown down by centrifuge in gm.	Solids in suspension in %	Solids in solution in %
100 cc. dialysed juice+2 gm. Na_2CO_3	10.14	0.2820	0.0368	13.0	87.0
100 cc. dialysed juice+1 gm. Na_2CO_3	9.87	0.2820	0.0502	17.8	82.2
Dialysed juice only	5.95	0.2820	0.2558	90.7	9.3
100 cc. dialysed juice+1 cc. lactic acid	3.26	0.2820	0.0254	9.0	91.0
100 cc. dialysed juice+2 cc. lactic acid	2.86	0.2820	0.0044	1.6	98.4

Samples of 5 cc. of dialysed juice alone, and with the addition of the above reagents in amounts comparable to those added in previous experiments, were whirled for six minutes with the rheostat governing the speed of the centrifuge (International No. 2) set at the 10th stop, after which the fluid portion was decanted and the residue dried and weighed. In Table XVI are shown the solids in suspension and in solution, at various hydrogen ion concentrations, as classified by this procedure.

Both of these reagents apparently dispersed the colloids, thus supporting our previous conclusion reached on other grounds. It is unlikely, however, that the actual dispersion was as great as the figures indicate, since the increased hydration of the colloids as the reaction moved away from the isoelectric point would increase the viscosity of the fluid and slow up the rate of settling of the particles. What part of the dispersion was due to the change in hydrogen ion concentration and what part to the specific effect of the reagent cannot now be stated. While it seems likely that hydrogen ion concentration was

mainly responsible, it must not be forgotten that in an earlier experiment lactic acid, in the presence of the natural electrolytes of fresh juice, while increasing both acidity and hydration, caused a decrease in dispersion, and in the last experiment potassium acid phosphate had the same effect in dialysed juice. In the present experiment, there appears to be a direct and logical relation between dispersion and hydration: though bound water was not determined, previous experiments left no doubt that it increased on both the acid and alkaline side of neutrality.

It must not be supposed, of course, that the solid particles in dialysed juice are sharply divided into those which settle under the influence of centrifugal force and those which do not. In the foregoing experiment, the whole of the fluid portion was decanted, but this varied markedly in concentration at different depths. A tube of dialysed juice of Marquis wheat leaves, without the addition of any reagent, was centrifuged as above, and the sedimentation and rarefaction of solid matter determined by making refractometric readings on portions pipetted from various depths. The results are shown diagrammatically in Fig. 13, and indicate that the particles in the juice were in various stages of aggregation.

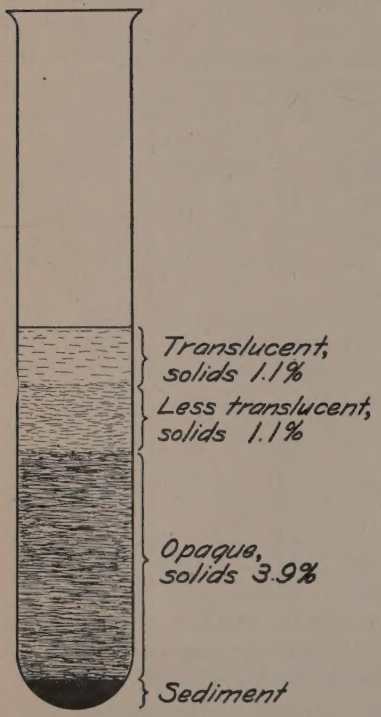

FIG. 13. *Stratification of solids of various degrees of dispersion under the influence of centrifugal force.*

The experiments recorded in this section had as one object the development of methods of studying the colloidal properties of plant-tissue fluids under controlled conditions. The extreme lability of these substances and the difficulty of making accurate comparisons of the fluids of different plants make such methods highly desirable. The investigations unfortunately had to be discontinued with the season of 1926, in which these experiments on dialysis were carried out. Their logical conclusion, the development of methods of study in which the natural properties of the colloids are fully preserved and by which they are measured under standard conditions with respect to hydrogen ion concentration and content of crystalloids, was not reached. However, the results already presented throw considerable light on hitherto unexplored physical properties of plant juices, and the possibilities and limitations of methods for their measurement.

Dialysis in distilled water, by the method described at the beginning of this section, was however used in a large number of routine determinations which

will be reported in the next section.

SUMMARY

The preparation of dialyser sacs of uniform permeability and an apparatus for continuous dialysis at low temperature are described. Cylindrical dialyser sacs of 100 cc. capacity were found to require about 48 hr. in which to effect satisfactory dialysis of plant juice at 3° C., with the water changed every hour. Larger sacs of globular shape were much less efficient.

A gradual, partial coagulation of colloids in plant juice takes place during dialysis, as shown by a decrease in the refractive index, by an increase in the gold number and, in one experiment, by sedimentation when centrifuged. This change was to some extent, but not entirely, prevented by dialysing in a 1% solution of common salt.

The hydration of the colloids in plant juice, as shown by the bound-water content, is frequently but not necessarily related to their state of dispersion. Both of these properties are affected markedly by hydrogen ion concentration, but may also be affected characteristically by certain salts. The addition of an acid or alkaline salt stimulates hydration, but not to an extent proportional to the change in hydrogen ion concentration, because of the opposing salt effect. In the presence of the natural crystalloids of plant juice, hydration is usually greater than in dialysed juice.

(*To be concluded in next issue*)